Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations

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Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations

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MODERN CONCEPTS IN BRAIN TUMOR THERAPY: LABORATORY AND CLINICAL INVESTIGATIONS

A Conference

held in

Atlanta, Georgia

February 26–28, 1976

Sponsored by:
Clinical Investigation Branch
and the
Cancer Clinical Investigations
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INTRODUCTION

The conference reported in this volume was designed by members of the Cancer Clinical Investigations Review Committee to bring together those working in the numerous areas of central nervous system tumor research in the hope that an exchange of information would be beneficial and lead to new avenues of research and treatment. Recent reports of brain tumor response to chemotherapy suggested that a new era of treatment might be on the horizon and, therefore, it is important that clinicians be brought up-to-date so that the promising preliminary results can be tested in well-designed clinical trials.

The conference was divided into four general areas: 1) fundamental therapy research including animal models and pharmacokinetics, 2) neuropathology and the correlation between humans and the animal models, 3) new methods of diagnosis including biochemical markers, and 4) methods of therapy including the analysis of response. That the aims of the conference were achieved is attested by the following presentations and discussions.

In the first session on research in therapy, Dr. Swenberg presented data on chemically and virally induced tumors in animals as excellent models in which to test chemical agents. He pointed out the extraordinary variation in the histology of the resulting tumor depending on the time during the antenatal period that the fetus was exposed to the agent. Dr. Wilson also discussed the tumor models in animals and their predictability in screening chemical agents. The discussion included comments about one model that demonstrated no significant effect of procarbazine, which apparently has significant antitumor effect in humans. Possibly, the dose employed was not high enough, since no great degree of toxicity occurred. Dr. Swenberg also warned that because many of the chemicals used in therapy are carcinogens, this fact should be weighed when their use is being considered. Considerable discussion was generated by Dr. Blasberg's presentation of the factors involved in the interactions between blood, cerebrospinal fluid (CSF), and brain, since these are vital to the success of chemotherapy. Some of the discussion also dwelt on the possible advantages of intra-arterial injections or infusions and treatment via the intrathecal route. Dr. Blasberg believed that the gains of such "localized" treatment with its diminished systemic toxicity rarely outweigh the disadvantages. For example, the tumors frequently derive their blood supply from more than one artery and the circulation time from the lumbar space is slow, so that drug concentration is greatly decreased by the time the cranium is reached.

Dr. Hoshino's research with thymidine uptake by brain and tumor cells pointed out some of the problems of diffusion. The discussion centered around the possibility that there are nonproliferating tumor cells that do not take up the thymidine and that also would be insensitive to cell cycle-specific agents.

In the session on neuropathology, Doctors Rubinstein and Vogel described the gliomas as a "biological continuum" varying from benign to highly malignant and noted that subdivisions tend to be arbitrary. Dr. Rubinstein commented upon the difficulty in relating brain tumors produced in the laboratory with those found in the human. He pointed out that we do not know what factors make for either rapid or slow growth of central nervous system neoplasms. During the discussion, Dr. Vogel and others speculated on Rubinstein's finding that the most dense area of tumor proliferation is frequently around areas of necrosis as if the central hypoxia stimulated the hyperplasia.

Doctors Caveness and Mahaley reported on the changes in tumors and normal brain in monkeys and man after irradiation and/or chemotherapy. It was believed that the delayed necrosis and demyelinization seen after X-ray therapy are preceded by fibrinoid necrosis of the blood vessel wall. Endothelial damage leads to changes in perfusion; members of the audience suggested that it might be advantageous to give chemotherapy concurrently with irradiation to take advantage of the changes in the blood–brain barrier.

Several members of the panel and audience discussed the effect of irradiation to the cranium on the immune response. The possibility was raised that such irradiation alters the brain's privileged immune status, allowing it to become more susceptible to viral infections. Doctors Chang and Mahaley discussed the existence of lymphopenia in patients with malignant gliomas prior to any treatment, and this lymphopenia probably is exaggerated by irradiation to the head.

Doctors Cooper and Kricheff presented data on the value and relative merits of nuclear scans and computerized axial tomography (CAT) with and without enhancement. These techniques mark an enormous advance in the diagnosis and accurate delineation of space-occupying lesions, but both agreed that the nuclear scan and, to a lesser extent, the CAT had limited usefulness in differentiating between tumor recurrence and postradiation changes. There seems to be little correlation between the appearance of a nuclear scan and the malignant potential of a glioma. Dr. Blasberg pointed out that nuclear scans are measuring changes in the blood–brain barrier. Such changes can be modified by concurrent therapy, especially with corticosteroids which, by their effect on diffusion, could lead to a false impression of improvement.

Information on biochemical markers in the CSF was presented by Drs. Ransohoff and Paoletti. Desmosterol and polyamines correlate well with the presence of a tumor, but the need for multiple CSF samples limited their usefulness in following the progression of the disease. Dr. Bogoch presented data on the preparation of two purified brain tumor antigens: astrocytin derived from human malignant glial tissues, and malignin from malignant glial cell cultures. With these two antigens, it was possible to demonstrate the presence of an antibody in the serum of most patients with malignant brain tumors; all but 4 elderly patients so tested demonstrated the antibody. Doctors Gutin and Chang discussed the effect of chemicals and physical agents (hyperbaric oxygen) on normal brain substance and tumors. The discussion highlighted the fact that insufficient attention is being paid to the use of combined-agent chemotherapy and that drug–drug and drug–irradiation interactions are probably underestimated. Dr. Ransohoff suggested that the corticosteroid effect on the basement membrane of blood vessels might well decrease the damaging effect of irradiation.

Dr. Bleyer presented excellent data on the levels of methotrexate in the CSF, brain, and tumors depending on the rate and route of administration. He pointed out the error of basing dose calculations on surface areas because the brain size and CSF bear little relationship to body surface area. The adult brain volume is reached early in childhood. Considerable discussion ensued on the merits and disadvantages of the intraventricular administration of methotrexate via the Ommaya device. High concentrations can be maintained, but obstruction to the CSF circulation can lead to levels that produce local brain necrosis. Responding to a question regarding the value of intrathecal therapy for tumors residing within the brain substance, Dr. Bleyer stated that methotrexate in the CSF does indeed penetrate the parenchyma to a level of millimeters or even 1 cm from the surface.

Dr. Gehan presented an analysis of the prognostic factors in the survival of patients with brain tumors. He sought to define significant parameters for stratification of patient groups. Numerous variables such as age, sex, pathology, site, and signs of cranial nervous involvement were evaluated as predictions of outcome. Although it was agreed that having comparable groups in clinical studies is vital, possibly Dr. Gehan's criteria can be used to develop historical control groups to eliminate the need for untreated control patients in each new study.

Dr. Wilson discussed problems in measuring response and documenting recurrent disease. Duration of response should be included as a measurement of a drug's effectiveness in phase II studies, whereas the time to recurrence is important in phase III. Every attempt should be made to document recurrent disease with the use of several methods such as neurologic examination and nuclear and CAT scans.

Dr. Walker presented the results of the study of primary brain tumors conducted by the Brain Tumor Study Group; the Radiation Therapy Oncology Group's work on tumors metastatic to the brain was discussed by Dr. Kramer. The conference closed with short presentations of eight studies being conducted by national cooperative units including the Southwest Oncology Group, Acute Leukemia Group B, European Organization for Research on Treatment of Cancer, Western Cooperative Oncology Group, Radiation Therapy Oncology Group, Eastern Cooperative Oncology Group, and the Children's Cancer Study Groups, many of which are only in the preliminary phases of patient entry.

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Chemical- and Virus-Induced Brain Tumors[1]

James A. Swenberg[2,3]

ABSTRACT—Experimental animal models resembling most human brain tumor types can be induced by exposure to oncogenic viruses or chemical carcinogens: Astrocytomas and glioblastoma multiforme can be produced experimentally by intracerebral injection of oncornaviruses, whereas medulloblastomas, choroid plexus papillomas, and ependymomas can be induced by the papovaviruses. Adenoviruses have been utilized to cause medulloepitheliomas, neuroblastomas, and retinoblastomas. All three groups of viruses can result in sarcoma production. Gliomas represent the primary tumor type induced in the brain by chemical carcinogens. These autochthonous tumor systems are reviewed, with emphasis on methods, tumor type, latency period, advantages, and disadvantages. In addition, recent investigations of molecular events involved in neoplastic transformation by chemical carcinogens are summarized.—Natl Cancer Inst Monogr 46: 3–10, 1977.

A variety of experimental brain tumor models resembling most human tumor types have been developed during the past decade. Brain tumors can be induced with intracerebral injection of viruses or chemicals, or by transplacental, parenteral, oral, or even topical exposure to chemical carcinogens. Depending on the model, such tumors arise weeks to years after exposure. The availability of suitable animal models has stimulated increased research on brain tumor kinetics, immunology, chemotherapy, and histogenesis. Recently, these models have been used in the study of molecular events responsible for neoplastic transformation. This review is intended to summarize the experimental brain tumor systems available today and to highlight some of the exciting research being done with these systems.

VIRUS-INDUCED BRAIN TUMORS

Work on experimental brain tumor induction with viruses was reviewed comprehensively in several publications (*1–6*). Intracranial tumors have been induced by representatives of most oncogenic tumor virus groups, including the adenoviruses, papovaviruses, and oncornaviruses. To date, techniques for induction and morphologic descriptions were emphasized in most studies on these models. Future investigations will probably de-emphasize morphologic classification and instead turn toward utilizing such models in basic and applied research. Morphologic studies have led to several generalizations that are summarized in table 1. For example, only oncornaviruses induce astrocytomas and glioblastomas, whereas the human papovavirus JC is the only virus known to cause the development of medulloblastomas. Choroid plexus papillomas are induced by simian virus 40 (SV40), the SV–PML papovaviruses isolated from patients with progressive multifocal leukoencephalopathy (PML) and the avian adenovirus (CELO), whereas medulloepitheliomas and retinoblastomas are only elicited with human adenoviruses. In contradistinction, sarcomas were induced by members of most tumor virus groups.

TABLE 1.—*Specificity of experimental brain tumor induction with oncogenic viruses*

Experimental brain tumor type	Inducing viruses	
	Group	Type[a]
Anaplastic astrocytoma or glioblastoma multiforme	RNA-Oncornaviruses	ASV, MuSV, SSV
Medulloblastoma	DNA-Papova	Human papova JC
Neuroblastomas or retinoblastomas	DNA-Adenovirus	Human adenovirus type 12
Ependymomas or choroid plexus papillomas	DNA-Papova	SSV40, human papova PML-1, JC, and BK
	DNA-Adenovirus	SA7 and CELO
Sarcomas or meningeal tumors	RNA-Oncornavirus	ASV and MuSV
	DNA-Papova	Bovine papilloma, murine polyoma, human papova JC
	DNA-Adenovirus	SA7

[a] ASV = avian sarcoma virus; MuSV = murine sarcoma virus; SSV = simian sarcoma virus; BK = human papovavirus BK; SA7 = simian adenovirus 7.

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Pathology and Toxicology Research Unit, The Upjohn Company, Kalamazoo, Mich. 49001.

[3] Dr. Darell Bigner provided many helpful suggestions during the preparation of this manuscript.

RNA Tumor Viruses

Avian Sarcoma Virus

Mammalian neuro-oncogenicity of ASV was first described by Rabotti and Raine (*7*). Subsequently, gliomas and intracranial sarcomas were induced in cats, dogs, guinea pigs, mice, rabbits, rats, and subhuman primates. The relative proportion of glial and mesenchymal elements in these brain tumors varies considerably among species. Homogeneous glial tumors have clearly been produced in the rat and dog with ASV. Bigner et al. (*8*) induced gliomas in 27 of 30 dogs, 19 of which had gliomas only, with no evidence of sarcoma formation. They later demonstrated that the site of ASV inoculation influenced tumor type; i.e., injection of ASV deep in periventricular zones resulted exclusively in astrocytomas, whereas only sarcomas were induced if the virus was injected superficially over the cerebellar vermis (*9*). Well-differentiated astrocytomas also developed in F344 (*10*) and Sprague-Dawley (*11*) rats after neonatal intracerebral injections of ASV. In addition, Copeland et al. (*12*) demonstrated that rats remained susceptible to the neuro-oncogenic effects of ASV at 100 days of age. Although the incidence of brain tumors in rats inoculated with ASV as adults was only 50%, nearly all were astrocytic.

In contrast to the rat and dog, a large percentage of the hamster, guinea pig, mouse, cat, and subhuman primate tumors induced with intracerebral ASV have a mesenchymal nature. Whereas neoplastic astrocytic elements can be observed, prominent reticulin fibers are also present. Tumors composed of large balloon-like cells (*13*) and "giant cell glioblastomas" (*14, 15*) usually contain such reticulin. This cell type probably represents a histiocytic element in a mesenchymal or mixed glial–mesenchymal tumor.

Several other features of ASV-induced brain tumors deserve comment: 1) There is generally no replication of infectious or noninfectious virus in mammalian tumor systems. However, virus structural antigens and virus-related transplantation antigens are usually produced in transformed mammalian cells. One can "rescue" ASV from such cells by growing the "nonpermissive" transformed cells with normal avian cells and allowing cell fusion to occur. 2) The dose of virus required to elicit intracranial tumors is lower than that for extracranial tumor induction. This is probably due to a greater immunologic response against the latter. 3) Improvements in the techniques for growing and concentrating ASV have allowed large pools of standardized virus to be produced (*16*). Using this virus, researchers have obtained reproducible survival curves for rats bearing a 100% incidence of astrocytomas. This system provides the first primary glioma model that is well suited for chemotherapy trials (*11*). Preliminary results of these trials have been reported (*5, 16–18*).

Other Oncornaviruses

MuSV has been reported to cause brain tumors in mice and rats following intracerebral inoculation (*5*). Gliomas, meningiomas, and hemangioendotheliomas represent the most common tumor types induced with MuSV. In contrast to ASV-induced mammalian tumors, replicating virions were easily detectable in these mouse tumors by electron microscopy (*19*). Recently, a 100% incidence of extremely vascular brain tumors containing neoplastic astrocytes and extensive endothelial proliferation was induced in Wistar-Furth rats with the Kirsten MuSV (*20*). No C-type virions were detected by electron microscopy.

Intracerebral inoculation of neonatal marmosets with SSV induced solitary tumors in 6 of 10 animals (*21*). The tumors were composed of pleomorphic neuroglial cells, areas of necrosis, hemorrhage, palisading, and prominent endothelial proliferation. These SSV-induced marmoset tumors closely resemble the human glioblastoma multiforme. Infectious virus was isolated from brain and cerebral spinal fluid of tumor-bearing animals.

DNA Tumor Viruses

Papovaviruses

Several members of the papovavirus group cause tumors when inoculated intracerebrally into animals (*5*). Bovine papilloma virus induced meningiomas, fibromas, and fibrosarcomas in hamsters and calves following latency periods ranging from 20 days to nearly a year. No virus particles were detected in the tumors by electron microscopy. Fibrosarcomas were also detected in the leptomeninges after polyoma virus was injected intracerebrally into newborn rats, hamsters, and rabbits. Intracerebral inoculations of SV40 into newborn hamsters induced fibrosarcomas in the leptomeninges and choroid plexus papillomas in the ventricles.

Of greatest interest, however, are the tumors that developed in hamsters after intracerebral injections of JC virus, a human papovavirus isolated from a patient with PML (*22–24*). Brain tumors developed in 50 of 63 hamsters inoculated with JC virus, with medulloblastomas representing the most common tumor type. These appear to arise from the internal granule cell layer of the cerebellum and frequently are multifocal. Several other tumor types have been induced with JC virus, including primitive gliomas, papillary ependymomas, meningiomas, and pineocytomas. Virus was recovered from 5 of 7 tumors tested. The JC virus is morphologically indistinguishable from SV40 and SV40-PML strain 1, but differs from them in its cell culture host range and antigenic properties (*5, 25*).

Adenoviruses

The experimental induction of neurogenic tumors in rats, hamsters, and mice after the animals received intracerebral, intraocular, or ip inoculations of human adenovirus 12 provides an excellent model for future research on neuroblastomas, medulloepitheliomas, and retinoblastomas (*26–31*). Medulloepitheliomas were induced in 88% of the rats given intracerebral injections of human adenovirus 12. These appeared to develop from the subependymal plate and were characterized by the formation of rosettes, high levels of cholinesterase following in vitro cultivation, and the presence of cilia containing 9+0 tubules (a hallmark of normal sensory neuronal cells). Similar 9+0 tubules were demonstrated ultrastructurally in the cilia of retinoblastomas induced in rats and hamsters following neonatal intraocular injections of human adenovirus 12.

Other members of the adenovirus group that elicited intracranial tumors (*5*) include the simian and avian adenoviruses (SA7, SV20, and CELO). SA7 induced poorly differentiated tumors of the choroid plexus, trigeminal nerve, and dura mater. Injection of SV20 into hamsters caused extremely undifferentiated tumors, which had the same morphology whether induced sc or intracerebrally. Intracerebral tumors produced with CELO had a morphology distinctly different from neoplasms induced with either the human or the simian adenoviruses; they were composed of papillary arrangements of cuboidal and columnar cells that filled the ventricles. Although originally thought to be ependymal tumors, the CELO tumors probably represent choroid plexus papillomas.

CHEMICALLY INDUCED TUMORS OF THE CENTRAL NERVOUS SYSTEM (CNS)

The susceptibility of neuroectodermal tissue to the oncogenic effects of chemical carcinogens was first demonstrated in 1939 (*32*). Since then, brain tumors have been induced by local implantation of several polycyclic hydrocarbons (PCH). More recently, additional classes of carcinogens were shown to be oncogenic for the nervous system following systemic exposure. These ranged from agents such as 2-acetylaminofluorene, which caused a few tumors of the nervous system along with many tumors elsewhere, to the nitrosoureas that caused neuroectodermal tumors in 100% of the animals. Several reviews (*33–36*) and a monograph (*5*) have been published on the chemical induction of brain tumors. In addition to reports by some scientists on the induction and characterization of various brain tumor models, other investigators used animal models for neuro-oncogenesis to study the molecular basis for neoplastic transformation. Whether similar mechanisms are involved in human brain tumors is unknown. It has been estimated that 80–90% of all human cancer is caused by chemicals; however, no chemical carcinogen has been clearly linked to human brain tumors.

In the section to follow, various brain tumor models have been arranged by the class of carcinogen used to induce them.

Polycyclic Hydrocarbons

Over 200 years ago, Sir Percival Pott first theorized the potential carcinogenic hazard of PCH. Therefore, it was fitting that the first experimental tumors of the nervous system were also induced with PCH (*32*). Since that time, brain tumors have been produced with several PCH, including 3-methylcholanthrene, benzo[*a*]pyrene, dibenzanthracene, dimethylbenz[*a*]anthracene (DMBA), and trimethyl benzanthracene. Such tumors have been induced in frogs, toads, mice, rats, hamsters, and dogs. However, birds, guinea pigs, rabbits, cats, and monkeys were resistant to the neuro-oncogenic effect of PCH. In general, brain tumors occur only when the PCH are in direct contact with the CNS. To accomplish this, the carcinogens are directly implanted in the brain. The incidence and type of tumor produced by PCH are greatly influenced by the positioning of the pellet (*37*). Few gliomas but many sarcomas and meningiomas result from superficial placement of the pellet near the dura. Ependymomas were primarily induced when the pellet was placed in the ventricles, whereas oligodendrogliomas were associated with implantation in the frontal white matter. Astrocytomas arose near pellets placed in the subcortical regions of the parietal lobe, and medulloblastomas formed after pellet implantation into the cerebellum.

Although several investigators have successfully induced brain tumors in rats with PCH, mice appear to be the most susceptible animal species (*34*). Tumor incidence varies from less than 10 to nearly 100%, with an average of 40–60% of the animals developing tumors. Most animals die between 200 and 300 days; however, latency periods have varied from as short as 68 to as long as 750 days. The primary advantage of this model is that the site of tumor formation can be localized. Disadvantages of the system include the variable incidence and latency period of the experimental tumors. The model is poorly suited for quantitative dose–response studies and investigations of early biochemical processes, since the time of exposure can vary from minutes to months because of the extremely long half-life of the pellet. The importance of unique doughnut-shaped virions detected in macrophages surrounding pellets of carcinogens prior to tumor formation requires further elucidation (*38*). Whether these virions represent activated viruses that play an important role in tumor induction remains unknown. However, they have never been detected in experimental gliomas and have induced fibrosarcomas rather than gliomas when they were injected intracerebrally into mice.

When pregnant rats were exposed to DMBA iv on day 21 (*39*) or orally on days 14, 17, and 20 of gestation (*40*), the most frequently detected neoplasms induced in the offspring were tumors of the nervous system. Transplacental exposure to additional PCH will be necessary to determine whether this is a general phenomenon or a specific effect of DMBA. In any event, it should now be possible to design pulse exposure experiments and to demonstrate dose–response relationships for DMBA.

Nitroso Compounds

A landmark in experimental neuro-oncology occurred in 1964 when Druckrey and co-workers (*41*) selectively induced tumors of the rat nervous system after iv injections of methylnitrosourea (MNU). This provided the first reproducible brain tumor model that did not traumatize the brain. Since then, several additional carcinogens have been discovered that primarily induce neuroectodermal tumors following systemic administration. These animal models represent some of the most powerful tools for modern research in chemical neuro-oncogenesis.

Methylnitrosourea

This acyl-alkyl-nitrosamide is among the most potent of all the chemical carcinogens known today. When administered iv at repeated low doses, the nervous system clearly represents the target organ. The highest incidence of neurogenic tumors is obtained with repeated iv injections of MNU at a dose schedule of 5 mg/kg/week for 32–36 weeks (*42*). This dosage has consistently produced a 90–100% incidence of grossly detectable brain tumors in Sprague-Dawley rats. Well-differentiated neurinomas are also induced; however, the incidence of these is much lower. This low incidence of tumors of the peripheral nervous system (PNS) can be reversed if F344 rats are used instead of Sprague-Dawley (*43*). The rat and the rabbit appear most susceptible to brain tumor induction with MNU (*5*). Dogs have been more variable; some brain and peripheral nervous system tumors have been induced in various breeds. Mice were quite resistant to neuro-oncogenesis with MNU. Denlinger et al. (*44*) induced 2 gliomas in 19 C3H mice exposed iv to 25 mg/kg every 4 weeks (total dose, 175 mg/kg). In spite of observation periods exceeding 6 years, attempts to induce brain tumors in rhesus monkeys have been unsuccessful (*5*). When cats were exposed to iv or oral doses of 25 or 20 mg/kg, respectively, all animals died within 10 days from severe bone marrow toxicity and septicemia (*35*). Additional resistant species include guinea pigs, sheep, and swine.

Anaplastic gliomas, mixed gliomas, and oligodendrogliomas represent the most common brain tumors induced with MNU. These are frequently located near periventricular regions, in subcortical white matter, and in the hippocampus. If the dose of MNU is raised from 5 mg/kg/week to 10 or 20 mg/kg twice a week, a shift from glial to mesenchymal cell types becomes apparent, with most tumors being sarcomas or gliosarcomas (*45*). MNU is also a potent carcinogen when administered locally, as shown clearly in experiments utilizing the sc, ip, or oral routes. Tumors develop at the site of administration and to a lesser extent in the nervous system. The lower incidence of tumors detected in the nervous system can best be explained by the decreased levels of circulating MNU. In addition to tumors at the site of injection and in the nervous system, a high incidence of thymic lymphomas can be induced after oral or sc administration of 20 mg/kg twice a week for 9 weeks. These types of experiments have clearly shown that MNU is not "neurospecific." Rather, MNU is a potent carcinogen for many tissues. Potential mechanisms involved in determining which target site is affected by this and related carcinogens will be discussed later.

Ethylnitrosourea (ENU)

The exquisite sensitivity of the fetal rat's nervous system to the oncogenic effects of ENU was first demonstrated in the experiments of Ivankovic and Druckrey (*46*). A 100% incidence of neurogenic tumors developed in offspring exposed to a single dose of 20 mg/kg or more during the last trimester of pregnancy. Clear dose–response relationships exist with single doses of 1, 5, 20, or 50 mg/kg inducing a 12, 79, 100, and 100% incidence of neurogenic tumors, respectively (*47*). While the tumor incidence increased, the latency period for tumor induction decreased from a mean of 655 days for 1 mg/kg to 211 days for 50 mg/kg. Age of the individual greatly influences the susceptibility to the neuro-oncogenic effects of ENU. The rat fetus is resistant to the carcinogenic effects of ENU before the 12th day of gestation; however, it is susceptible to teratogenic effects before this stage of development. Susceptibility to neuro-oncogenesis increases from the 12th day of gestation to birth, after which it begins decreasing (*33*). By 30 days of age, the rat has a susceptibility comparable to the adult; i.e., the nervous system no longer represents the target organ and much higher doses of ENU are required to induce a similar incidence of neoplasia.

ENU has usually been administered iv to pregnant rats, although it can be given orally, ip, or sc. Neurogenic tumors were even reported in 38% of the offspring whose mothers had 50 mg/kg of ENU applied topically to the skin (*48*). It has also been possible to induce tumors by administration of precursors of ENU in the food and water of pregnant rats. Under the mildly acidic conditions of the rat's stomach, ethylurea and sodium nitrite undergo nitrosation and form ENU in vivo (*49*).

Rats are the species most sensitive to the neuro-oncogenic effects of ENU. Hamsters, mice, and opossums have also developed neurogenic tumors. In hamsters, tumors have been confined to the PNS, whereas in mice a low incidence of CNS and PNS tumors develops. Mixed gliomas and oligodendrogliomas represent the most common brain tumors induced with ENU, whereas anaplastic neurinomas are the type occurring most frequently in the PNS. Anaplastic gliomas and sarcomas are rare. Animals bearing anaplastic neurinomas die significantly earlier than those bearing gliomas. When the sequential development of these tumors was investigated, evidence of neoplastic proliferation was detected in trigeminal nerves as early as 3 weeks after exposure to ENU (*50*). Comparable changes were not evident in the brain until 4 months after ENU exposure. Mechanisms involved in this differential triggering of neoplastic proliferation between glial and Schwann cells remain unknown.

Other Nitroso Compounds

Additional nitroso compounds possessing neuro-oncogenic properties have been described since the original discovery of MNU (*5, 33*), including dimethylnitrosourea, trimethylnitrosourea, propylnitrosourea, butylnitrosourea, dinitrosopiperazine, nitrosopiperidine, nitrosomorpholine, ethylnitrosobiuret, methylnitrosobiuret, ethylnitrosourethane, and methylnitrosourethane. In general, these offer no major advantages over MNU and ENU and will not be discussed in detail.

Other Carcinogens

Triazenes

Several of the dialkyl-aryl-triazenes have been shown to be carcinogenic for the nervous system as well as for other tissues (*51*). Neuro-oncogenesis has been successful both in adult rats and transplacentally. As noted for MNU and ENU, the methyl compounds were most effective in adults, whereas the corresponding ethyl compounds were highly oncogenic to the fetal rat's nervous system.

1,2-Diethylhydrazine, Azoethane, and Azoxyethane

Transplacental neuro-oncogenesis has been induced in the rat with these three carcinogens (*33*). In contrast to the triazenes, adult rats developed multiple intestinal tumors instead of neurogenic neoplasms when the methyl counterpart was administered.

INVESTIGATIONS ON THE MECHANISMS OF CHEMICAL NEURO-ONCOGENESIS

Scientists conducting studies at several laboratories have suggested a possible mechanism for the neurospecificity of some chemical carcinogens. That chemical carcinogens are electrophilic reagents has been known for several years (*52*). Those carcinogens that are not electrophilic must be metabolized to form electrophiles, which interact with macromolecules such as DNA, RNA, and protein. Neoplastic transformation represents a heritable change at the cellular level, with the most likely site for this change being the DNA of the originally transformed cell (*53*).

Investigations of several carcinogens and tissues demonstrated alkylation of DNA bases. The greatest amount of this alkylation occurred at the N-7 position on guanine, with lesser amounts being detected at the N-3 position of adenine and the O^6-position of guanine. When the extent of *N*-7 alkylguanine formation was determined in brains and livers of adult and 10-day-old rats given injections of ENU, no correlation was found between DNA alkylation and carcinogenicity (*54*). Subsequent studies determined the degree of purine alkylation at various times after ENU exposure. Goth and Rajewsky (*55, 56*) demonstrated surprising differences between the repair rates of O^6-ethylguanine and those of *N*-7 ethylguanine or *N*-3-ethyladenine. O^6-Ethylguanine persisted for long periods in the target organ (brain) but was repaired much more rapidly in the liver. *N*-7-Ethylguanine was removed rapidly in both tissues. If one determines the brain:liver ratio for each of the major sites of purine alkylation, a high degree of correlation is evident between alkylation at the O^6-position of guanine and carcinogenicity.

Further support for this hypothesis has been reported by Kleihues and co-workers (*57–59*), who demonstrated persistence of O^6-methylguanine in brains of rats treated with MNU and methylmethanesulfonate (MMS). Both methylating agents caused brain tumors in rats; however, MMS has a much weaker neuro-oncogenic effect. When equimolar doses of MNU and MMS were administered to rats, only 0.05 as much O^6-methylguanine was present after exposure to MMS. Thus the extent of initial O^6-alkylguanine formation and its persistence over time correlates with carcinogenicity. A possible mechanism for this correlation was suggested by Loveless (*60*). Alkylation at the O^6-position of guanine forces the base into the enol form, which causes anomalous base pairing during DNA replication. Instead of pairing with cytosine, O^6-alkylguanine pairs with thymine. Fixation of anomalous base pairing requires that at least one cell replication take place before repair of the damaged DNA. The lack of this replication in end-stage neurons may explain why no neuronal tumors have been induced with MNU, even though DNA alkylation occurs to a greater extent in neurons than in glia (*61*). It also helps to explain the predilection sites for ENU- and MNU-induced gliomas, since these areas contain the glia with the greatest propensity for cell replication. Enzymic repair systems for O^6-alkylguanine have recently been demonstrated (*62, 63*). If these systems are deficient in rat brain, the exquisite susceptibility of the fetal rat brain to carcinogens may thus be explained. Over half the 35 or more compounds that caused transplacental carcinogenesis induced tumors of the rat nervous system.

In summary, pathogenetic mechanisms involved in experimental neuro-oncogenesis seem to be as well or better known than those of other neoplasms. Delayed repair of O^6-alkylguanine appears to be a major factor in sensitizing the rat nervous system to carcinogenesis. Whether this mechanism is responsible for human brain tumors remains to be investigated. Sensitivity to neuro-oncogenesis may be a general phenomenon for many carcinogens if effective tissue concentrations reach the nervous system. At present, the somatic mutation theory of cancer induction best explains chemical neuro-oncogenesis.

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Animal Models of Brain Tumors [1, 2]

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ABSTRACT—Although no single model of the numerous animal models of brain tumors developed in recent years perfectly represents the spontaneous human tumors, different ones do have specific advantages for certain types of studies. Chemical induction in adult animals, transplacental chemical induction, viral induction, and transplantation are effective methods that allow choice of numerous histologic types of tumors which, to a greater or lesser extent, simulate human tumors. Reproducibility of location, cell type, and time of tumor appearances; expense; ability to grow in tissue culture; safety for personnel; trauma to brain; nature of vasculature, and amount of brain and tumor tissue available for examination are among the variables to be considered in choosing a model appropriate for a particular study.—Natl Cancer Inst Monog 46: 11–17, 1977.

As chemical oncolytic agents came into use in the 1940's and early 1950's, the National Institutes of Health Screening Committee established a panel of animal tumor models. It was soon apparent that standard models could not be used to predict the potential effectiveness of brain tumor drugs; compounds such as nitrogen mustard, potent against extraneural tumors, proved ineffective in clinical trials. Then in 1962, Chirigos et al. (*1*) demonstrated that cyclophosphamide was effective against mouse L1210 leukemia if the tumor implant were placed subcutaneously but ineffective if placed intracerebrally. To complicate the situation even more, Shapiro (*2*) later showed that implants of VM-26 inhibited tumor DNA production whether they were placed in the brain or subcutaneously. VM-26 also inhibited growth at both sites, although a subcutaneous tumor developed less than an intracerebral one. However, VM-26 did not improve survival in animals bearing the brain tumor (*2*).

The importance of cellular growth kinetics suggested that glial tumors would predict response of human glial tumors better than would high growth fraction tumors such as L1210 leukemia, Dunning leukemia, B16 melanoma, or Ehrlich carcinoma, even though the efficacy of 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU) was originally demonstrated on intracerebral L1210 (*3*). Whereas it was unlikely that any given model could perfectly simulate all human brain tumors, physiologically and in response to any treatment, tumors of brain tissue were essential. Furthermore, since the vasculature of a tumor originating from brain differs depending on whether it is placed in brain or subcutaneous tissue, such a tumor model should be located in the brain (*4*). To be useful in evaluating drug and other therapies, such a model should also be consistent in tumor type from animal to animal and predictable in disease onset and course (table 1).

TABLE 1.—*Types of studies*

1. Drug screening and evaluation
   a. Individual drugs, dose schedules, and combinations
   b. Radiation therapy schedules
   c. Immunotherapy trials
2. Pharmacokinetics
3. Tumor-cell population kinetics
4. Immunology
5. Biochemistry, blood–brain barrier

In 1939, Seligman and Shear (*5*) demonstrated that 3-methylcholanthrene implanted in the brains of mice induced neuroectodermal tumors. This was repeated by Zimmerman and Arnold in 1941 and studied subsequently (*6*, *7*). Several other polycyclic hydrocarbons, such as dibenzanthracene, benzo[*a*]pyrene, 7,12-dimethylbenz[*a*]anthracene (DMBA), and trimethylbenzanthracene, also induced tumors similarly. Susceptibility to tumor induction varied; mice, frogs, hamsters, and dogs were susceptible, whereas birds, guinea pigs, rabbits, cats, and monkeys were not (*8*). Some mouse strains proved more susceptible than others. The type of tumor induced varied; in general, implantation of the pellet or powder superficially in the brain caused sarcomas and meningiomas, whereas placement in frontal white

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matter elicited a high percentage of oligodendrogliomas. Subcortical parietal lobe placement produced many astrocytomas, cerebellar placement induced medulloblastomas, and intraventricular placement gave rise to ependymomas (*7*).

Latency periods varied from 68 to 750 days, with a tumor incidence of approximately 40–60%. Obviously, this makes evaluation of any treatment difficult because the tumor is not apparent until a near lethal mass has been attained, and the mortality distribution is so scattered that statistical evaluation is nearly impossible. This model has allowed study of several interesting issues in tumorigenesis, such as the presence of virus particles (capable of producing fibrosarcomas on reinjection) in macrophages near the chemical implant shortly before, but not after, the tumors develop (*9*). These neoplasms also have the disadvantage of a traumatic introduction of the inciting agent at the site of the tumor, which may alter tissue character and vasculature.

In 1964, Druckrey et al. (*8*) and Swenberg and co-workers (*10–13*) found that methylnitrosourea (MNU) injected iv would cause a high incidence of neural tumors. Repeated iv doses weekly for 32–36 weeks can produce tumors in as many as 90–100% of the Sprague-Dawley rats. In general, rats and rabbits have been most susceptible, with dogs, mice, cats, and monkeys much less so. This carcinogen produces various tumors, including anaplastic gliomas, mixed gliomas, and oligodendrogliomas, although at higher dose levels, an increasing incidence of sarcomas, gliosarcomas, and peripheral nerve tumors such as neurinomas occur. These tumors also have cell-cycle time and histologic similarities to human tumor types. Again, there is variability between strains because F344 and Sprague-Dawley rats are more likely to develop neurinomas and gliomas, respectively (table 2).

ENU given to a pregnant rat in the last trimester by virtually any route will cause a high incidence of nervous system tumors in the offspring (*14, 15*); unlike MNU, it has little central nervous system oncogenic effect on adult rats, which tend to develop leukemias instead (*12*).

Induction with transplacentally administered ENU commonly produces mixed gliomas and oligodendrogliomas, as well as anaplastic neurinomas of the fifth cranial nerve, whereas anaplastic gliomas and sarcomas are rare. Anaplastic ependymomas occur in the spinal cord. This model has the advantage of inducing tumors with single injections in animals of the same age, thereby saving the considerable effort of multiple iv administration needed with MNU and allowing more precise estimates of neoplastic onset.

TABLE 2.—*Classification of models*[a]

Type	Chemical	Viral
Autochthonous	Polycyclic hydrocarbons 3-Methylcholanthrene and others MNU given iv to adult rats, rabbits ENU given transplacentally to rates	ASV, MuSV, SV40, Human JC papovavirus Human adenovirus 12
Transplanted	3-Methylcholanthrene-induced ependymoblastoma, glioma 26, glioma 261 Rat MNU-induced tumor lines, e.g., 9L	ASV-induced gliosarcoma in beagles

[a] ASV = avian sarcoma virus; MuSV = murine sarcoma virus; SV40 = simian virus 40; ENU = ethylnitrosourea.

The advantage of the MNU and ENU models is the production of tumor types similar to human gliomas without mechanical trauma to the brain. Disadvantages are: 1) lack of localization of the tumor, 2) inconsistency of histologic tumor type, 3) multiplicity of tumors, and 4) the induction of tumors outside the nervous system; also, 5) some scatter in tumor induction times (and hence of survival) is present. Nevertheless, some chemotherapy experiments have been performed. For example, Swenberg (*16*) was able to demonstrate some increase in survival time of MNU-treated rats that had received 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU).

A number of different compounds have been used similarly; these include other nitroso compounds, triazenes, 1,2-diethylhydrazine, azoethane, and azoxyethane, all of which have little advantage over MNU and ENU as chemical carcinogens.

Variability of tumor type and unpredictability of time of tumor onset and death can be avoided by the direct implantation of tumor cells or fragments. Since 1891, many attempts have been made to implant human tumors in the brains of various animals, with mixed success. It has usually proved difficult to maintain serial transplantation, but even when transplantation is successful fairly consistently, such as that of human choriocarcinoma in the monkey brain, the xenogeneic nature of the tumor creates several new variables. Notable examples are host-immune response and differences in the nature of the supporting stroma. Consequently, conclusions based on these models are difficult to generalize (*17*).

TABLE 3.—*Applications of models*

Method	Advantages	Disadvantages	Major use at present
3-Methyl cholanthrene implant	Economical Type of tumor controllable to a degree by site of chemical implant	40–60% incidence of tumors Latency long and variable Variety of tumors	
MNU to adult rats	No trauma to brain Histology and cell kinetics similar to human tumors	Laborious induction Multiple tumors and tumor types Only fair standardization of temporal course of disease	Biochemistry Peritumoral edema Immunology
ENU transplacentally	No trauma to brain Histology and cell kinetics similar to human tumors Less work than MNU	Multiplicity of tumors and tumor types Only fair standardization of temporal course of disease	Chemical carcinogenesis Biochemistry Immunology
Mouse ependymoblastoma lines (transplanted)	Economical Reproducible Standard tumor, predictable Statistically amenable time course	Trauma to brain Small brain Relationship of tumor model to human tumors questionable histologically and by cellular kinetics criteria	Chemotherapy screen
Transplanted MNU-induced tumor	Moderately expensive Reproducible Tissue culture available	Trauma to brain Uncertainty as to neuroglial nature of tumor	Chemotherapy Radiotherapy Evaluation for cell-kill
Virus-induced tumors (ASV)	Less trauma to brain Autochthonous tumor with natural blood supply Fairly long but predictable course Cell kinetics similar to human	Multiplicity of tumors and tumor types Expense and care of working with viruses	Immunology Chemotherapy Viral tumorigenesis
Transplantable virus-induced tumor (only one in use is the beagle)	Large brain for blood–brain barrier, pharmacokinetic studies Consistent tumor type	Trauma to brain Expensive for studies requiring large number of animals Question whether blood–brain barrier of dog similar to that of human	Pharmacokinetics Chemotherapy

More useful has been the serial transplantation of nervous system tumors, initially induced by a chemical carcinogen, into successive members of the same inbred animal strain. This procedure promotes selection of the cells most resistant to host defenses and produces fairly rapidly growing, consistent tumors. The mouse ependymoblastoma cell lines, including ependymoblastoma A, glioma 26, and glioma 261, have been carried subcutaneously for many generations in C3H and C57BL/6 mice. Unlike many other tumors, they have remained histologically stable under these conditions. The tumor can be removed when desired, cut into small pieces that are drawn into a spinal needle and, after the skull is punctured, extruded into the brain by a stylet. Alternatively, the tumor can be dispersed in a balanced salt solution, and a brei of cells can be injected into the brain. As many mice as desired can receive implants in this way; nearly all will develop neoplasms of the same type and die in 2.5–3.5 weeks (*18, 19*).

This model has been used extensively in evaluating drugs (table 3). For example, ependymoblastoma A has been the basis of extensive testing by the Drug Evaluation Branch of the National Cancer Institute. With the use of multiple dosage levels of each drug tested, as well as several dosage schedules, groups of treated mice can be compared with untreated mice. For the more effective compounds, a percentage of long-term survivors can be obtained; e.g., 78% of mice treated daily for 5 days with BCNU were long-term survivors, as were 86% with CCNU. A more generally useful measure of comparison is to take the day of death of the median survivor in each group of test animals. The median life-span of the treated animals minus that of the controls, divided by the median life-span of the controls, gives the increased life-span.

The results of these studies allow ranking of the effectiveness of a large number of drugs and hence an indication of their probable effectiveness in human patients (*20*). One of the problems of such models is illustrated when treatment is started on day 1; the tissue and vascular disruption from tumor implantation are unlike conditions in a spontaneous human tumor, and the cells are still contained in a piece of tissue without its own blood supply; therefore, drug delivery and cell kinetics are likely to be unrepresentative.

Furthermore, the ependymoblastoma is a rare tumor type in man. Indeed, the relevance of the mouse ependymoblastoma to the human is unclear because the mouse cells lack many of the ultrastructural characteristics of ependymal cells (*21*), and the cell kinetics are unlike those of human tumors (*13*). As it happens, dianhydrogalactitol, the most effective drug in these experiments, is much less effective against mouse glioma 26 or rat 9L gliosarcoma (*22*). Although the prediction of effectiveness for the nitrosoureas and procarbazine is confirmed in human experience (tending to affirm the validity of the model), the use of dianhydrogalactitol (which scientists are only beginning to introduce in clinical trials) may demonstrate some of the limitations of the model.

Just as the 3-methylcholanthrene-induced mouse ependymoblastoma can be used in serial transplantation, so the nitrosourea-induced tumors can be transplanted within the same strain (usually rats). Whereas the rat model has the disadvantage of greater expense for experiments with large numbers of animals, it has the advantage of a larger brain for those involving examination of tumor and brain, such as pharmacokinetic studies. In addition, the rat gliosarcoma produced by MNU can be grown in tissue culture. This permits maintenance stocks to be kept frozen for future use of the same transplant generation; it also allows good single-cell suspensions for quantitative tumor inocula (*23*) and in vitro evaluation of cell viability before and after treatment by the colony assay system (*24*). Finally, the transplanted rat tumor is easy to use and provides another species in which one can check drugs screened in the mouse, since no single model is likely to be a perfect predictor of human tumor response to anticancer drugs.

The major disadvantages of a transplanted tumor are: 1) The transplant is introduced via traumatic means just before a period of tumor proliferation, and 2) several tumor lines have shown a tendency to develop a more sarcomatous and less glial appearance, whereas the mouse lines have remained histologically more stable. Gliofibrillary protein and S-100 protein are produced in some lines under certain conditions [Rubinstein L, Kornblith P: Personal communication; (*25*)].

Recently, another approach to tumor induction has been virus inoculation. The oncogenicity of Rous sarcoma virus has been known since 1912. In the last few years, various strains of ASV, such as the Bryan, the B77, and the Schmidt-Ruppin, have caused tumors of mammalian tissues. The type of tumor in the brain, as with 3-methylcholanthrene, depends on the location of inoculation (*26*). A superficial cerebral or cerebellar vermian location favors development of sarcomas. Inoculation in the subependymal plate area produces a high incidence of gliomas. F344 and Sprague-Dawley rats have developed a high incidence of astrocytomas. Many of these tumors include cells with the appearance of gemistocytes, although in the hamster, cat, and monkey, these cells seem to be producing reticulin, which raises some question about their parentage (*13*).

A pool of virus can be stored frozen for 5–10 years without loss of oncogenicity, and a measured quantity can be injected into the brain. In a rat inoculated neonatally, the tumors develop in about 4 weeks and cause death in about 3 months (*16*).

The resulting tumor has the blood supply of a spontaneous tumor; because a smaller needle and inoculum can be used, the trauma of inoculation is less, and also more distant in time, compared with a transplanted tumor. Yet the location of the tumor, its time of development, and death of the host are fairly predictable. Furthermore, the cell cycle time is similar to that of human tumors, and the course is long enough for delayed toxicity of drugs and immune response of the host to be evaluated. Tumors may be multiple and of multiple types, including a significant incidence of sarcomas.

Swenberg et al. (*16*) used the B77 strain of ASV in Sprague-Dawley rats with treatment beginning at 1 month, by which time control rats had 2-mm astrocytomas. When BCNU and CCNU were given iv every 3 weeks, significant extension of life was seen; the results with BCNU were superior to those with CCNU. Mithramycin, given ip five times every 3 weeks, was less effective; these results are consistent with human phase III trials of the Brain Tumor Study Group in which BCNU has shown effectiveness and mithramycin did not. However, with BCNU and CCNU, 20–60% of the rats died of sarcomas (frequently located in the thalamus) that apparently developed later than the astrocytomas but were more resistant to chemotherapy. Hence the effect of the nitrosoureas on glial tumors may be greater than suggested by the raw survival figures. This model showed little efficacy of procarbazine, which has been effective in human phase II trials (*16*).

Swenberg's group has also used this model to evaluate PCNU [1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea], a new more lipid-soluble nitrosourea, and preliminary results are promising; this correlates with more extensive evaluations of PCNU in the transplantable rat gliosarcoma by Levin (*27*). The same model is proving especially useful for immunologic studies because of the longer life-span of the animals and the decrease in abnormal vascular reaction at the initiation of the tumor.

Many other viral models have been developed but they seem to have greater limitations. For example, human adenovirus 12 causes neural tumors in several animals, such as mouse, hamster, and rat (*28–31*). In the rat, medulloepitheliomas, medulloblastomas, ependymoblastomas, and embryonic gliomas are produced. However, the time of death is variable, and this model may be more useful for cytokinetic and immunologic investigations than for chemotherapy.

The JC papovavirus, isolated originally from a patient with progressive multifocal leukoencephalopathy, produces brain tumors in 83% of hamsters 4–6 months after neonatal inoculation (*32*); fairly typical medulloblastomas are produced as well as glioblastomas and primitive tumors. Unfortunately, the virus is difficult to maintain.

SV40- and polyoma virus-transformed cells have produced ependymomas and choroid plexus papillomas (*33*). Simian adenovirus SA7 in the hamster induced several tumors, including choroid plexus papillomas and medulloblastomas; the latter have now been grown in tissue culture and transplanted (*34, 35*). The Moloney strain of murine sarcoma virus (M-MuSV) will cause malignant astrocytomas in rats (*36–38*), but this is a replicating virus system in which the virus persists in the tumor. Recently, Yung et al. (*39*) demonstrated that the Kirsten strain of murine sarcoma virus (Ki-MuSV) has produced highly malignant gliomas in young rats inoculated intracerebrally. Tumors induced by the Ki-MuSV appeared consistently after about 16 days, caused death fairly uniformly at 4–5 weeks, and produced a consistent type of neoplasm, notable for marked vascular proliferation. Virus particles have not been seen on electron microscopy, but this may merely indicate a low rate of virus production. Further studies are in progress to determine whether complete virions, capable of infectivity, persist and replicate in this model tumor.

One of the major drawbacks of the viral systems is the need for viral facilities and considerable care; the SV40 and the JC papovavirus are potentially hazardous to man. Less stringent precautions are needed for ASV than for the others, since mammalian cells are not permissive for ASV. Although viral genome can be demonstrated, and virus can be produced by transfer back to the avian host, replication of intact virions does not continue in the tumor as it does in the one induced by M-MuSV. With its consistent results, the ASV now seems the best choice for chemotherapeutic and immunologic studies of virus-induced tumors, although the Ki-MuSV model may be of similar value, if the tumor is shown not to sustain viral replication.

Like the chemically induced tumors, those elicited by viruses can be transplanted within an inbred strain. This has been exploited by Wodinsky (*40*), who used inbred beagle puppies to produce a larger animal model. About 90% of dogs inoculated develop tumors, which often were pilocytic astrocytomas. Wodinsky initially transplanted a beagle gliosarcoma sc into an irradiated pup; from the third transplant generation on, the inoculations were intracerebral. The histology remained constant for 22 generations. The tumor cells can be stored at −195° C for later use (*40*).

The tumor was inoculated into the right frontal lobe of beagle puppies less than 24 hours old. Median survival time initially was 40 days but when this experiment was repeated in subsequent generations, it decreased to 13 days. Studies in this model have shown the effectiveness of BCNU and CCNU, but not of cyclophosphamide, which again correlates well with clinical experience.

For chemotherapy survival studies, the canine model is quite expensive, since so many dogs are required for statistical validity. Furthermore, survival is short. However, the dog does provide a much larger amount of brain tissue, especially white matter and tumor, for evaluation of fluid, electrolyte, and chemical changes, as well as pharmacokinetic parameters.

Clearly, none of these models is ideal for all purposes. Most have the tumor arising in mechanically traumatized brain. Several involve glial tumors not really typical of any common human tumor, and several have changed their character with time. Many are too variable for statistical analysis. Smaller animals have little brain tissue for pharmacokinetic and biochemical studies. Yet each model has certain advantages. The final test of a model is whether it accurately represents a human tumor for the parameter being tested, whether that be disturbance of blood–brain barrier or response to chemotherapy. For the most part, chemotherapy studies in these models have agreed with human experience, although some useful drugs might have been missed by one or another assay. To date, animal studies have frequently followed rather than preceded the comparable human studies. It appears unlikely that a single perfect model, appropriate for all aspects of the variety of human brain tumors, will be developed in the foreseeable future. More likely, individual investigators will continue to choose one or more models with those advantages that conform to their particular needs.

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Pharmacodynamics and the Blood–Brain Barrier[1]

Ronald G. Blasberg[2, 3]

ABSTRACT—The optimal delivery of a drug(s) to the brain or to a brain tumor is dependent on the unique anatomy of the brain and on a number of interdependent pharmacokinetic and physicochemical parameters. The parameters that affect drug activity at the target site are divided into three phases: 1) administration, 2) distribution (delivery), and 3) biologic (mechanisms of action/metabolism/excretion). Each of the parameters must be considered with respect to three levels of possible influence or control: 1) clinical, 2) pharmaceutical, and 3) physiologic. Clinical control of drug activity at the target site is mediated primarily through factors that affect drug administration. Drugs can be delivered to the brain (tumor) by three separate routes: 1) systemic (iv), 2) intra-arterial, and 3) intrathecal. The most important factors affecting iv drug administration are the time course of drug action, the rate of biotransformation, metabolism, and excretion, and the permeability of brain and tumor capillaries. Local chemotherapy by intra-arterial administration has an advantage over the iv route only if the time course of drug action is short (does not exceed the time course of intra-arterial infusion), and if a significant fraction of the drug is taken up by the brain (tumor) or biotransformed/metabolized/excreted during first passage through the brain and lungs. The most important factors affecting intrathecal administration and diffusional transport into the brain from the cerebrospinal fluid (CSF) are a long time course of action; rapid equilibration within, but slow clearance from, the CSF; very slow rates of biotransformation; and a very low capillary transfer constant.—Natl Cancer Inst Monogr 46: 19–27, 1977.

This presentation will attempt to develop certain guidelines for more effective central nervous system (CNS) chemotherapy from the viewpoint of pharmacokinetics and drug delivery to the target site. The guidelines should be useful to the clinician treating patients with a tumor of the CNS and to the research worker involved in the evaluation of specific drugs and drug protocols. This discussion will not present specific supporting data; the experimental studies are described elsewhere (*1–4*) and were reviewed at a recent workshop (*5–7*).

In many ways, the delivery of chemotherapeutic drugs to the brain and to other organs is similar. However, two anatomical features of the brain make this organ unique: 1) the tight junctions of the endothelial cells of the cerebral capillaries and 2) the ventricular and subarachnoid cerebrospinal fluid (CSF).

The tight junction of the endothelial cells of the capillaries is the apparent anatomical basis for the blood–brain barrier, which retards the rapid equilibration of large, polar, lipid-insoluble compounds between blood and brain tissue extracellular fluid (ECF). Nonpolar, lipid-soluble drugs exchange rapidly across brain capillaries (as well as other biologic membranes). A similar blood–CSF barrier is also present, but the tight junctions appear between choroidal epithelial cells rather than between choroidal endothelial cells.

The CSF circulates over the large surface area of the brain and provides an alternative route for drug delivery. The CSF can function as a "source fluid" and as a "reservoir" of a drug after intrathecal administration; a drug will diffuse from CSF across the ependymal (or pia-glial) surface into and through the brain ECF down its concentration gradient. Under certain conditions after systemic administration of a compound, the CSF could function as a "sink" for parenchymal drug or drug metabolite. These considerations may be of considerable importance to the distribution of a compound between blood, brain, and CSF.

The sequence of steps that affect drug activity in brain tissue or a CNS neoplasm may be divided into three general phases: 1) drug administration, 2) drug distribution (delivery), and 3) biologic effect (therapeutic/toxic). These phases may be considered separately with respect to three levels of influence, the first of which concerns the clinician or researcher; i.e., how can he effectively influence the delivery of more drug to a CNS

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[3] Dr. David P. Rall initiated many of the ideas expressed in this manuscript at the NCI in the early 1960's; more recently, Drs. Allen Cowles and Walter Eckman have made significant contributions. Dr. Clifford Patlak (National Institute of Mental Health) has consistently given aid and provided useful discussion. Particular appreciation is expressed to Dr. Joseph D. Fenstermacher, who provided excellent guidance and in whose laboratory this work is being continued. The skilled secretarial assistance of Mrs. Emily Olausen and Mrs. Josephine Clem is gratefully acknowledged.

TABLE 1.—*Parameters affecting drug activity in brain and CNS neoplasms*

Steps	Clinical	Pharmaceutical	Physiologic
Administration	Drug Site Dose Rate Schedule		
Distribution			Bulk flow of blood/CSF
		Physical characteristics	Binding to serum proteins
		"	Capillary permeability
		"	Diffusion through ECF
		"	Distribution in tissue fluids
		"	Tumor/cellular uptake
Biologic effect (therapeutic/toxic)	Inhibitors/competitors	Pharmacologic characteristics	Mechanism of action Metabolism/excretion

tumor and increase the therapeutic benefit without increasing the toxic effects? The second level of influence, the pharmaceutical, deals with the importance of physical characteristics as well as pharmacologic considerations in the design and development of new antineoplastic drugs. The third refers to the physiologic and biochemical parameters that determine the distribution (delivery), the biologic activity, and the metabolism and excretion of a drug once it has been administered. The clinician and pharmacologist can do comparatively little to influence these physiologic and biochemical parameters, which, as properties of a biologic system, have to be understood and accepted to optimize the administration schedule for a particular drug.

Table 1 outlines the sequence of steps and the parameters that influence drug activity in the brain (or brain tumor). The subsequent discussion will attempt to integrate the various parameters that affect drug activity into a composite picture of CNS chemotherapy. The physician and the researcher may gain a better understanding of the complexities of chemotherapy of the CNS and will recognize certain guidelines useful in the clinic and laboratory.

DRUG ADMINISTRATION

The physician has direct control over several parameters that provide an opportunity to maximize drug delivery to the target site and to minimize systemic toxicity. The parameters, outlined in table 1, are listed sequentially below.

The choice of drug(s) is obviously important. Occasionally, the choice of an effective drug is inappropriate for a particular mode of administration or the mode of administration is inappropriate for the choice of a particular drug; that is, one may observe a therapeutic failure with an effective drug if that drug is administered in the wrong way.

Drug administration may be systemic or local; the sites of systemic administration, i.e., iv, im, sc, or oral, will be considered collectively, since the distribution of drug is essentially the same for these various routes.[4] Local administration may be accomplished intra-arterially and intrathecally. Although the local route is technically more complex and involves increased risk to the patient, it may yield a significant therapeutic-to-toxic advantage under certain conditions.

The dose or amount of drug administered depends on several other variables including the rate and/or schedule of administration. Methotrexate illustrates the importance of this dependence; a single dose of 350 mg/kg is required to produce a lethal effect (LD50) in rats, whereas less than 2.4 mg/kg total dose infused over 96 hours produces a similar effect (*8*).

The rate of drug administration, from a bolus injection to a constant infusion (or perfusion), is controllable. Under certain conditions, it may be desirable to maintain serum or CSF levels constant for a particular period rather than have a peak concentration followed by rapidly falling drug levels (*9*). The advantage of a constant infusion over bolus administration was clearly shown for cytosine arabinoside; an infusion is necessary to maintain adequate blood levels of this cell cycle-specific drug for a particular period because of its rapid deamination and loss of activity (*10*).

Multiple-dose scheduling can be considered a more convenient form of administration to maintain relatively constant blood (or CSF) drug levels in comparison to a constant infusion (or perfusion). Another and more important aspect of multiple-dose scheduling relates to the importance of normal cell and tumor cell growth kinetics and to the optimum time between sequential courses of chemotherapy. (This will be discussed in the next paper.)

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[4] This is true provided there is no local metabolism at the site of administration or no significant digestive tract or hepatic metabolism after the drug is given orally or ip.

DRUG DISTRIBUTION

The distribution of a drug throughout the various fluid and tissue compartments in the body depends primarily on the physical characteristics of the compound and on certain physiologic processes. The clinician has limited influence on these processes or parameters. However, the chemist may be able to alter the physical characteristics of the drug to obtain a more favorable distribution (delivery) at the target site without sacrificing its therapeutic effect. This was recently demonstrated for a polar cholinesterase reactivating drug; a nonpolar transient derivative of the active drug was synthesized that readily permeated brain capillaries and, once within the brain, was rapidly oxidized to the active quaternary salt (*11*). Table 1 sets out several of the parameters that affect the distribution of a drug in the body.

The drug is distributed within the fluid to which it has been administered (blood or CSF) and is delivered to the target tissue by bulk movement. Bulk flow of blood throughout the body and to the capillaries of the target organ occurs in seconds. Recent studies have demonstrated surprisingly rapid bulk movement (circulation) of CSF as well; the time course varies from minutes to several hours (*12*).

The binding of a drug to serum proteins (primarily serum albumin) is particularly important and has been recognized for many years (*13–15*). The drug–protein complex effectively reduces the activity or concentration of "free" drug and serves as an exchangeable reservoir of drug. Since albumin appears to be excluded from the CNS (CSF to serum albumin concentration ratio is less than 1:100), considerably less total drug will appear in the CSF than in the serum if a significant fraction of the drug is bound to serum proteins. However, the activity of the drug is a function of its free or unbound concentration. Therefore, we should be more concerned with the ratios of unbound drug between various fluid and tissue compartments. The CSF-to-serum (or brain-to-serum) concentration ratios commonly measured may reflect the effects of serum protein binding more than the effects of impermeable brain capillaries. This binding has been noted for several anticonvulsant drugs and has been studied extensively; e.g., diphenylhydantoin is lipid soluble and 94% bound to serum albumin (*16, 17*). The unbound serum fraction closely correlates with the CSF concentration. Therefore, the CSF-to-serum concentration ratio of total diphenylhydantoin reflects the fraction of unbound to bound drug more than the impermeability of brain capillaries.

The transport of most drugs from blood to brain ECF (or vice versa) across the endothelial cells of CNS capillaries appears to be passive and restricted for most polar, lipid-insoluble compounds. The "restricted" transcapillary movement of a drug in the brain, however, is a relative process; the transcapillary flux in brain is less than that observed in most other tissue. Brain capillaries do not establish an absolute barrier; most compounds will permeate the capillaries to some finite extent. The permeability of brain capillaries to a particular drug may be expressed in terms of a "transfer constant," i.e., the amount of drug that enters the brain during a particular period of time. An equilibrium will eventually be established. If the brain capillaries have a small but finite permeability with respect to a particular drug and if the serum concentration of that compound is kept constant, the amount of drug in the brain ECF will eventually equilibrate with that in the serum so that equal concentrations (activities) could exist in serum and brain ECF. If the drug in the brain ECF is removed by a mechanism such as cell uptake, metabolism, or biotransformation, and diffusion into the CSF sink, the brain ECF-to-serum concentration ratio will be low and will reflect the ratio of the capillary permeability (transfer) constant to the rate constant of drug removal from the ECF. Therefore, if the capillary permeability constant is low and the rate constant for the removal of drug from the ECF is high, the transport of drug across the endothelial cells of CNS capillaries could be a rate-limiting step. However, if the rate constant for drug removal from the brain ECF is also low, the brain ECF-to-serum concentration ratio at equilibrium may not be too far from unity. In this instance, satisfactory brain levels could be reached over a long time course (provided that serum levels are maintained), despite the fact that the capillary permeability constant is low.

The passive transport of nonpolar, lipid-soluble drugs across the endothelial cells of brain capillaries is usually considered to be "unrestricted;" significant differences between the brain and other organs are not apparent. Since the capillary permeability constant is relatively high for these drugs, capillary exchange is not usually considered to be a rate-limiting step. However, if biotransformation or cell uptake and metabolism of the drug from the ECF is rapid, a low ECF-to-serum concentration ratio could be observed. The point I wish to stress is that the ECF concentration of drug in the brain or in a CNS neoplasm is due to the rate constant of ECF removal of drug (cell uptake, metabolism, biotransformation, and/or diffusion into the CSF sink) as well as to the capillary permeability constant of the drug.

A drug moves through the normal brain predominantly by diffusion. The aqueous diffusion constant of a drug is a physical characteristic of the molecule and is a function of its molecular size. The aqueous diffusion constants of most drugs are between 5 and 7×10^{-6} cm^2/second at 37° C. For comparative purposes, the aqueous diffusion constants of sodium and serum albumin are 20×10^{-6} cm^2/second and 0.7×10^{-6} cm^2/second, respectively, at 37° C. The rate of drug movement through the brain is a function of its apparent diffusion constant in brain tissue. The diffusional movement of drug through the brain ECF may be measured; an apparent diffusion constant in brain caudate nucleus has been obtained for several chemotherapeutic drugs (*2*). The apparent diffusion constant of a polar, lipid-insoluble drug in the brain will always be less than the diffusion constant of the drug in water because of several factors: 1) the tortuosity of the ECF pathways; the microscopic diffusional distance around cells is always longer than the macroscopic diffusional distance directly through the tissue, and 2) the effect of cell uptake and/or binding, diffusion into dead-end pores and drug metabolism or biotransformation will reduce the amount of drug in the ECF; therefore, less drug remains free in the ECF to diffuse further into the brain, and the observed or apparent diffusion constant will effectively be reduced (*2*). The diffusional transport of a nonpolar, lipid-soluble drug that rapidly equilibrates across cell membranes may not be limited to the ECF; diffusional transport may also occur through cells. When this occurs, the apparent brain diffusion constant of the drug may approach its aqueous diffusion constant.

Bulk flow of drug entrained in the ECF could enhance or retard the diffusional movement of drug through the ECF, particularly if the drug is diffusing into the brain from a CSF surface. Our unpublished studies in the normal rhesus monkey indicate that little or no bulk flow of ECF occurs; however, it is possible that significant bulk flow does occur in or around tumor tissue with abnormal capillaries and edema fluid.

The distribution of drug within the various tissue fluids as well as the brain and CSF is also important with respect to systemic toxicity. The unique relationship between the brain and the CSF is especially relevant to CNS chemotherapy. The CSF can act as the source fluid for intrathecal drug administration and as a reservoir or "depot" of drug for diffusional transport into brain tissue. A drug that has been administered into the CSF will also be cleared from this compartment and enter the systemic circulation and could result in systemic toxicity (*18*). In the patient receiving systemic therapy in whom low drug levels are maintained in the CSF compared with brain ECF, the CSF could act as a sink with drug diffusing from brain ECF to CSF.[5] Drug distribution depends partly on the physical characteristics of the drug; therefore, some pharmaceutical influence can be exercised.

The movement of a drug across normal and tumor cell membranes, either by diffusion or by specific transport processes, and the distribution of drug within the various organelles of the cell cytoplasm and nucleoplasm are complex processes. The intracellular distribution is more closely related to the molecular biology and biochemistry of drug action (*20, 21*). Selective uptake of chemotherapeutic drugs by malignant tissue could enhance or raise the therapeutic effects with little or no increase in systemic toxicity.

Now that we have completed a simplified review of the parameters that affect drug delivery to the target site, I want to discuss and compare the specific advantages for each of the three routes of drug administration (iv, intra-arterial, intrathecal) in greater detail. Certain guidelines will be introduced for the clinician or researcher: 1) the most important parameters affecting drug delivery by each of the three routes of administration and 2) the most effective time course of administration for a drug with a particular set of physicochemical characteristics.

SYSTEMIC (iv) ADMINISTRATION

Some of the most important parameters affecting the iv administration of a drug and the delivery to the target site within the CNS, outlined in table 2, are: 1) the time course of drug action required to produce the desired effect at the target site, 2) the rate of drug biotransformation, metabolism, and excretion at the target site as well as in the systemic circulation, and 3) the

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[5] The relative importance of the "sink effect" of the CSF, with respect to electrolyte concentrations in the ECF at various distances within the brain, was recently analyzed (*19*); the analysis is also relevant to other compounds when the parenchymal ECF levels are higher than those of the CSF.

TABLE 2.—*Parameters affecting systemic drug administration*[a]

Parameters[b] and administration	Effect							
Parameters								
Time course of action	Short				Long			
Metabolism/excretion	Slow		Rapid		Slow		Rapid	
Capillary permeability	High	Low	High	Low	High	Low	High	Low
Administration[a]								
Time course	Bolus	(Bolus)	(Infusion)	Infusion	(Bolus)	(Infusion)	Infusion	Infusion
Efficacy	Very good	Fair	Good	Poor	Very good	Good	Fair	Very poor
Model drugs[c]	—	—	BCNU Thio-TEPA (PCZ)	Mustard	—	MTX	(PCZ)	Ara-C

[a] *Parentheses* indicate a less clearly defined administration time course or model drug classification.

[b] Parameters that are less important include tumor (cell) uptake, diffusion in the ECF, and binding to serum proteins.

[c] BCNU = 1,3 bis(2-chloroethyl)-1-nitrosourea; MTX = methotrexate; PCZ = procarbazine; Ara-C = cytosine arabinoside; thio-TEPA = triethylenethiophosphoramide.

TABLE 3.—*Parameters affecting the intra-arterial to iv advantage*[a] *of drug administration*[b]

Parameters[c]	Effect							
Time course of action	Advantage[a] only during the time course of drug administration							
Metabolism/excretion	Rapid				Slow			
Capillary permeability	High		Low		High		Low	
Capillary blood flow	Low	High	Low	High	Low	High	Low	High
Advantage[a, b]	Very good	(Very good)	Good	(Good)	Fair	(Fair)	Poor	(Poor)

[a] The intra-arterial to iv advantage is expressed in terms of their respective concentration × time $(c \times t)$ product in the brain or in a CNS neoplasm.

[b] *Parentheses* indicate a slightly lower advantage due to the higher capillary blood flow and an extraction fraction less than 1.0.

[c] Parameters that effect optimal drug delivery but not the intra-arterial to iv advantage; less important parameters include binding to serum proteins, tumor (cell) uptake, and diffusion in the ECF.

permeability of brain and tumor capillaries to the drug. Although some parameters are less important (*see* footnote *b*), they could become significant under certain conditions. For example, diffusion through ECF may limit drug delivery to neoplastic cells if the rates of biotransformation, cell uptake, and metabolism are rapid. As discussed earlier, significant protein binding will reduce the amount of free drug and could create a reservoir of exchangeable drug.

A compound is usually cleared from a particular tissue or fluid compartment through several independent processes often considered together and expressed as a clearance half-time (or as several clearance half-times). The chemotherapist's objective is to maintain an adequate concentration of drug in the serum and at the target site for a particular period; therefore, the time course of administration of a particular compound should reflect its clearance half-time and the time course of its action at the target site. For a drug with a long clearance half-time (slow biotransformation, metabolism, and excretion), a single bolus could provide adequate levels for a period of effective action. On the other hand, a drug with a short clearance half-time (rapid biotransformation, metabolism, and excretion) may require a constant infusion to provide adequate levels for the time course of drug action.

TABLE 4.—*Parameters affecting intrathecal administration*[a]

Parameters and administration	Effect			
Parameters				
Time course of action	Only for a period of hours to days			
CSF clearance	Slow		Fast	
Capillary permeability/metabolism/(cell uptake)	Low	High	Low	High
Administration efficacy	Very good	Poor	Fair	Very poor
Model drugs	MTX Ara-C	—	—	BCNU Thio-TEPA

[a] Parameters are those affecting intrathecal chemotherapy of parenchymal brain tumors.

The "efficacy rating" in tables 2–4 is at best an approximation and provides a general summary of the multiple parameters involved. The reader should not attach undue value to this scale because all the parameters may not be weighed equally for a particular drug; therefore, comparisons between different drugs are difficult to make. Similarly, the placement of specific "model drugs" within a particular category is an oversimplification. All too frequently we do not have quantitative measurements for the parameters involved or the measurements are incomplete, hence only a few specific examples or recommendations can be offered at this time from the viewpoint of pharmacokinetics. The nitrosoureas, thio-TEPA, and PCZ should be effectively delivered to a CNS neoplasm after systemic administration. Depending on their time course of action at the tumor site and their rate of clearance from the serum (biotransformation, metabolism, and excretion), a brief infusion or several shortly spaced bolus injections may provide more effective drug levels at the target site than a single bolus injection. MTX could be effectively delivered to a CNS neoplasm after systemic administration, even if the permeability of the surrounding capillaries were low, provided serum levels were maintained by repeated administration or by a constant infusion over a comparatively long time. Ara-C is rapidly deaminated after systemic administration; therefore, adequate serum levels of this drug can only be maintained by a constant infusion.

INTRA-ARTERIAL (LOCAL) ADMINISTRATION

Local intra-arterial drug administration to a tumor has been considered to have an advantage[6] over the systemic route, but most of the clinical studies conducted have not justified the additional risk nor the added technical expense (*22*). In several studies, specific criteria under which intra-arterial administration may have an appreciable advantage over that of iv have been developed (*3*, *4*). The first two parameters listed in table 3 are critical and were derived from these studies.

First, the intra-arterial technique has an advantage over the iv only during the time course of drug administration and for a short time thereafter, during which brain (or tumor) drug levels are decreasing to equilibrate with systemic serum levels. Stated another way, there is an intra-arterial advantage only during the initial passage of drug through the brain (tumor) capillaries; the concentration of drug in the blood during the second and subsequent passages through the brain (tumor) capillaries will be similar to that achieved by iv administration. Second, the rate of biotransformation, metabolism, and excretion during the first passage through the brain (tumor) and the lungs is important. The more rapid the degradation and excretion of drug during the first passage, the less drug will enter the systemic circulation and the lower the systemic toxicity will be for a given amount of administered drug. Stated in another way, for the same degree of systemic toxicity, considerably larger amounts of drug can be delivered to the brain (tumor) by the intra-arterial route (in comparison to the iv) if the rates of biotransformation, metabolism, and excretion during the first passage are high.

The rate of blood flow through the infused capillary bed is less significant; blood flow affected the intra-arterial-to-iv advantage for drugs that have extraction fractions below 1.0 (when the uptake by brain and/or tumor tissue is not blood-flow limited). With a lower rate of blood flow, there is a greater fractional uptake of drug (higher extraction fraction) by the brain (tumor), and less drug enters the systemic circulation. Unfortunately at the present time, the clinician has no way to control blood flow through tumor capillaries.

The other parameters explained in footnote *c* of table 3 do not specifically increase the intra-arterial-to-iv advantage (they affect both routes of drug administration equally) but have an important bearing on the maximum delivery of drug to the brain (tumor). In intra-arterial administration, the time course of drug action and the permeability of brain capillaries are probably the most important of these parameters.

Briefly, an "ideal" intra-arterial drug has the following characteristics: 1) The time course of drug action should be short, certainly not longer than that of intra-arterial infusion; 2) a significant fraction of the drug should be taken up by the brain (tumor) or be biotransformed or metabolized during the first passage through the brain (tumor) capillaries or be excreted by the lungs. These processes will reduce the amount of drug delivered to the systemic circulation and correspondingly reduce systemic toxicity. 3) The physical characteristics of the drug should be such that it rapidly equilibrates across brain (tumor) capillaries and across cell membranes. Neither the pharmaceutical industry nor most drug-screening programs are looking specifically for an "ideal" intra-arterial drug, particularly with respect to uptake by the brain (tumor). Indeed, most of the drugs that have been injected or infused intra-arterially were chosen because they demonstrated a significant effect after systemic administration. If we wish to use the intra-arterial route for local chemotherapy, we should seek a new class of drugs. Several highly labile drugs have demonstrated a selective advantage for intra-arterial infusion (*23–25*). The rapidly acting and degraded alkylating agents and the nitrosoureas are probably closest to the ideal intra-arterial drug at the present time.

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[6] The "advantage" of intra-arterial over iv administration may be expressed in terms of the ratio of their peak concentrations or in terms of the ratio of their $c \times t$ products at the target site (brain, tumor, or site of systemic toxicity such as the marrow or gut). The peak concentration will always be higher for the intra-arterial route and will primarily be a function of the total dose and rate of administration. In contrast, the $c \times t$ product more closely reflects the time course of an effective concentration of drug at the target site. Therefore, this discussion will use the word "advantage" to mean the ratio of the $c \times t$ product for the intra-arterial over the iv route of administration.

INTRATHECAL ADMINISTRATION

The rationale frequently cited for intrathecal chemotherapy is that direct administration into this compartment can achieve significantly higher concentrations of certain drugs within the CSF (and surrounding brain tissue) than the systemic route (*26*). The parameters that influence drug distribution after intrathecal administration have recently been reviewed (*27, 28*); table 4 shows some of the parameters that affect the movement of drug from CSF into the brain.

The time course of drug distribution within and between various CSF compartments ranges from several minutes to several hours (*12*), whereas the distribution of drug within the brain may take several hours to several days (*6*).

The rate of drug clearance has a significant effect on the brain tissue concentration profiles as well as on CSF levels (*5*). CSF drug levels can be maintained by ventriculolumbar perfusion, slow intraventricular infusion, or sequential bolus administration. Another effective way to maintain CSF drug levels and to increase the concentration of certain drugs in brain is to reduce the clearance of drug from the CSF compartment. In the case of MTX, CSF clearance may be reduced by: 1) blocking choroidal uptake and clearance with transport inhibitors such as probenecid and 2) reducing bulk CSF turnover or clearance by reducing CSF production with acetazolamide or cardiac glycosides (*29, 30*).

The diffusional movement of drug through the brain was discussed earlier; the rate of diffusional movement through the brain is a function of the apparent diffusion constant of the drug in brain. The loss of drug from the ECF by exchange or mediated transport across the capillary endothelium will reduce the amount of it in the ECF and could significantly retard its diffusional transport further into the brain. This limitation may also apply at the tumor site, even for drugs that normally do not exchange readily across brain capillaries. If the capillaries within the tumor are progressively more permeable to the drug, a significant amount could be lost across the altered capillaries and this loss could severely limit its diffusion to the central portions of the tumor. However, a recent study indicates that the capillaries at the periphery of the tumor (the site of most rapid tumor growth) remain impermeable to polar, nonlipophilic compounds (*31*).

The biotransformation and/or metabolism and the cell uptake and sequestration of drug will have an effect similar to that of transcapillary loss on the ECF concentration profile of the administered form; i.e., less drug will remain in the ECF for diffusion further into the brain. Once a steady state is reached, in which the amount of drug diffusing from the CSF into and through the brain ECF is balanced by the amount of drug being lost from the ECF by transcapillary loss and/or by biotransformation and/or cell uptake and metabolism, maintaining constant CSF drug levels will increase neither the concentration in nor the penetration of drug into the brain. Therefore, a drug which 1) readily permeates brain (tumor) capillaries, 2) is rapidly biotransformed, or 3) is taken up by cells and rapidly metabolized to an inactive compound, will never achieve effective tissue concentrations more than several millimeters from the CSF surface.

A recent study (*2*) has shown that a drug may be cleared from the ventriculocisternal perfusate (CSF) by diffusion into the brain parenchyma and by subsequent diffusion across parenchymal capillaries into the systemic circulation. The perfusate (CSF) clearance was significantly greater for those compounds with high capillary transfer constants (BCNU and thio-TEPA) than for compounds that did not permeate brain capillaries readily (MTX, hydroxyurea, and ara-C). Similarly, the biotransformation and the cell uptake and metabolism of a drug will increase its clearance from the CSF compartment.

The most important characteristics of an ideal drug for intrathecal administration and for diffusional transport into the brain parenchyma would be the opposite of those for the ideal drug for intra-arterial administration. The drug should have: 1) a comparatively long time course of action; 2) a rapid equilibration within, but slow clearance from, the CSF compartment; 3) a slow (insignificant) rate of biotransformation and/or metabolism; and 4) a low (insignificant) capillary transfer constant. These criteria (table 4) suggest that MTX, hydroxyurea, and ara-C have several distinct advantages over BCNU and thio-TEPA for intrathecal chemotherapy of parenchymal brain tumors.

If intrathecal chemotherapy is primarily directed against malignant cells dispersed within or lining the CSF compartment rather than against malignant cells within the brain parenchyma, the conditions for optimal drug delivery are somewhat changed. The parameters affecting diffusional transport of a compound from the CSF into and through the brain to the tumor site become less important. The primary factors now become: 1) the rate of mixing and the distribution of drug within the various CSF compartments; 2) the rate of drug clearance from the CSF; and 3) the time course of drug action. These parameters are quite interdependent as illustrated below. Effective therapy may be achieved with BCNU or thio-TEPA at the site of intrathecal administration, since the time course of drug action is probably short in comparison to the rate of clearance of drug from the CSF. However, it appears doubtful that these two chemotherapeutic agents would be distributed rapidly enough within all the various CSF compartments to ensure an adequate concentration of active drug throughout the subarachnoid space. Perhaps a rapid perfusion of the subarachnoid spaces with BCNU or thio-TEPA would be more effective.

A NOTE OF CAUTION

This discussion has focused on the various parameters affecting the pharmacokinetics of drug delivery to the target site; the mechanism of drug action (therapeutic and toxic) was not discussed. The guidelines developed for iv, intra-arterial, and intrathecal drug administration presumed that the compound is active at the target site. The guidelines indicate: 1) the most important parameters affecting delivery to the target site, 2) the optimal physical and pharmacologic characteristics for a particular mode of therapy, and 3) the schedule or time course of administration required to achieve an optimal concentration and time course at the target site. The examples involving specific drugs must be viewed with some caution. Many of the pharmacodynamic parameters cited have not been quantitated in animals or in humans, even with such well-studied compounds as methotrexate and the nitrosoureas; more research is needed in this area.

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Therapeutic Implications of Brain Tumor Cell Kinetics[1, 2]

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ABSTRACT—Cell kinetic studies on malignant human gliomas suggest that these tumor cells have a cell cycle time of 2–3 days, a duration of DNA synthesis phase of 7–10 hours, and an average labeling index of 5–10% with growth fraction of 0.3 in viable areas. From these results, the author concluded that 1) a single short-term course of a cell cycle-specific chemotherapeutic agent alone will probably fail to achieve either significant reduction in tumor mass or dramatic clinical improvement; 2) cell-cycle phase-specific drugs should be administered over 2–3 days to maintain an effective blood level for maximal tumor-cell kill. Preliminary analyses of isolated nuclei from brain tumors by flow microfluorometry (FMF) revealed a homogeneous, slowly proliferating population in meningiomas and low-grade astrocytomas. In contrast to these tumors, glioblastoma multiforme and anaplastic astrocytomas consisted of a more rapidly proliferating population with tetraploid and/or octaploid cells and also demonstrated a considerably varied population in each piece analyzed from a single tumor. This fact suggests that these tumors are much more heterogeneous with respect to population kinetics than either meningiomas or astrocytomas. Our ability to rapidly assess the population kinetics of human brain tumors with FMF may enhance the effectiveness of their clinical management.—Natl Cancer Inst Mono 46: 29–35, 1977.

The brain is a unique organ from the point of view of cell kinetics because neurons become incapable of cell division shortly after birth. The glial cells in the supportive tissue of the brain retain their proliferative capacity, as shown by reactive and reparative gliosis (*1*), but it is currently believed they do not proliferate at the rapid rate observed in other tissues. Several reports suggest that a low level of mitotic activity is continually present in glial populations of the adult mouse (*2*) and rat brain (*3, 4*). Interestingly enough, the cell cycle time of renewing glia is approximately 18–20 hours (*2, 4*), a value close to the cell cycle time of other regenerating systems. Although the growth fraction of glial tissue is extremely low, i.e., 0.004 (*2*), this slow production of new cells is enough to balance glial cell loss. The kinetic state of normal brain clearly contrasts with that of brain tumors, which consist of a more actively cycling population that is derived mostly from glial cells.

Kinetic data on normal human glia are not available, but incidental examination of unaffected brain from tumor-bearing patients to whom [^{3}H]thymidine had been administered revealed almost no labeled cells, which suggests low proliferative activity (*5*).

CELL KINETICS OF HUMAN GLIOMAS

The first in vivo study of a human glioblastoma was performed in 1960 by Johnson et al. (*6*), who injected multiple doses of [^{3}H]thymidine iv into a terminal patient and calculated a labeling index of 0.6%. A labeling index, which is the proportion of cells labeled shortly after a flash of [^{3}H]thymidine, represents the percentage of cells in DNA synthesis preparing for mitosis in relation to the total cell population. Therefore, it gives an approximation of the proliferative activity of the tissue being studied. Chigasaki (7) studied the in vitro uptake of [^{3}H]thymidine in biopsy specimens of a glioblastoma, an astrocytoma, and an oligodendroglioma, obtaining labeling indices of 0.94, 0.44, and 0.33%, respectively. Assuming the duration of S-phase to be 6–7 hours, they estimated the generation time of glioblastomas to be about 1 month. A few years later, Kury and Carter (*8*), using a method almost identical to Chigasaki's, examined the labeling index of several gliomas in vitro and reported that 2 glioblastomas had labeling indices of 3.6 and 6% and 5 anaplastic astrocytomas had labeling indices from 2 to 7%, all considerably higher values than those reported by Johnson et al. and Chigasaki. They estimated generation times of glioblastomas and malignant astrocytomas to be 3–5 and 2–10 days, respectively. Using local injection of [^{3}H]thymidine into glioma tissue at the time of craniotomy, Fukuma et al. (*9*) reported labeling indices similar to those obtained by Kury and Carter.

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Tym (*10*) introduced the stathmokinetic method; he added to the flash labeling technique by using vinblastine sulfate, which facilitates kinetic analysis by arresting and holding cells in metaphase. Studying one glioblastoma, Tym obtained a labeling index of 1.6% and calculated an S-phase of 8 hours and a cell cycle time of 5–10 days.

During the past few years, we studied 12 human malignant brain tumors, administering [^{3}H]thymidine iv during operation (*5*). From radioautographic study of these tumors, we observed that the labeling index varied not only among gliomas but also among different areas of the same tumor. However, the labeling index of a particular area correlated reasonably well with its histologic characteristics, although the relationship was not a strict one. For example, the labeling index within necrotic areas was nearly zero, whereas the index within the viable parts of the same tumor might approach 20%. By means of a double radioautography technique, we also estimated the duration of S-phase for each tumor.

Table 1 summarizes these kinetic parameters for 12 brain tumors. By our method, glioblastomas had an average labeling index of 5–10% and the value for astrocytomas was 1% or less. The labeling index of anaplastic astrocytomas fell between that of well-differentiated astrocytomas and glioblastomas. Within anaplastic astrocytomas, areas containing well-differentiated astrocytes, with or without microcysts, had a labeling index close to that of more organized astrocytomas, whereas areas containing anaplastic astrocytes with neoplastic vascular hyperplasia had a labeling index approaching that of similar areas within glioblastomas. Predominantly pilocytic areas in some glioblastomas possessed a far higher labeling index than was observed for otherwise identical pilocytic areas within astrocytomas. This suggests that despite morphologic similarities, the pilocytic astrocytes in glioblastomas are biologically different from pilocytic astrocytes in differentiated astrocytomas.

TABLE 1.—*Labeling index and duration of S-phase in brain tumors*

Case No.	Pathology	Labeling index, %	Duration of S-phase, hr	Birth rate[a]	Turnover time, hr[b]
1	Glioblastoma	5.9	5	1.23	79
2	"	11.4	7	1.71	59
3	"	8.1	—	—	—
4	"	5.5	10	0.59	191
5	"	4.4	9	0.49	203
6	Malignant astrocytoma	5.4	11	0.51	198
7	" "	8.2	13	0.65	156
8	" "	2.3	7	0.33	307
9	Astrocytoma	1.0	—	—	—
10	"	0.8	10	0.09	1,154
11	Metastatic	6.3	7	0.92	108
12	"	13.6	—	—	—

[a] Birth rate=No. of new cells/100 cells/hr.

[b] Turnover time=100 cells/birth rate.

In contrast to the variability of the labeling index in different astrocytic tumors, the duration of S(DNA synthetic)-phase remained within a range of 7–10 hours. Several factors may influence the duration of S-phase in vivo, but the uniformity of the S-phase in astrocytomas of widely differing malignancy indicates that degree of differentiation or dedifferentiation is not one of these factors.

With the factors obtained from the duration of S-phase and the labeling index, we can calculate the "birth rate" of the tumor, expressed as the number of newborn cells per 100 tumor cells per hour. A reciprocal number of the birth rate gives us "turnover time," i.e., that time required for the tumor to replace its whole population. For malignant gliomas, the turnover time is approximately a few days to a week, and in one astrocytoma it approached 2 months. Multiplying the turnover time by the conversion factor of 0.7 (*11*) yields the potential doubling time, which is the time required for a tumor population to double its size without cell loss.

By this method of calculation, the theoretical doubling time in glioblastomas is approximately 7 days; but every neurosurgeon knows that a glioblastoma does not double its size every week. The probable explanation for this discrepancy is that cell loss occurs simultaneously with cell growth in glioblastomas.

Growth fraction and cell cycle time are the most important parameters for understanding the basic proliferative activity of tumor cells and are also highly relevant to clinical treatment.

Table 2 shows the growth fraction and cell cycle time that we derived for 4 human glioblastomas (*12*) when Mendelsohn's method (*13*) and the stathmokinetic analysis originally designed by

TABLE 2.—*Growth fraction and cell cycle time in recurrent glioblastoma*[a]

Patient No.	Growth fraction[b]	Cell cycle time, hr
1	0.32 (0.10)	55.4 (11.5)
2	0.39 (0.10)	64.1 (12.9)
3	0.29 (0.07)	—
4	0.14 (0.06)	52.3 (26.5)
Average	0.28 (0.10)	57.3 (6.1)

[a] *Numbers in parentheses* indicate SD.

[b] Growth fraction was derived in viable area.

Puck and Steffen (*14*) were used. Methodological factors that modify the results (*15*) should be kept in mind when interpreting these data, although we obtained a mean growth fraction of approximately 30% and a cell cycle time of 2–3 days. I should note that the areas of tumors examined were confined to those parts that showed no microscopic necrosis and that were fairly evenly labeled. Thus the growth fraction would be smaller if we considered the whole tumor, which includes a large nonviable portion. If half the tumor is necrobiotic, a reasonable estimate in these glioblastoma patients, the growth fraction would be reduced by half. In this case, the growth fraction of glioblastomas might be 15% rather than the calculated 30%.

Even though considerable variability exists in the cell cycle times calculated from different samples of each tumor, the average values for 3 tumors were similar. When combined they gave a mean of 57.3 hours with an SD of 6.1 hours. In contrast to this agreement in cell cycle times, the growth fraction in patient #4 is markedly lower than for the other tumors. This same patient had a brain tumor diagnosed 9 years before this study. This pretreatment interval is much longer than in the other cases and demonstrates a good correlation between the smaller growth fraction and the slower growing neoplasm. From these data, it appears that the low labeling indices observed in slow-growing brain tumors may occur as a result of a low growth fraction and not merely as a reflection of a prolonged cell cycle time.

The cell cycle time of 2–3 days, though considerably shorter than that reported by others (*6*, *7*, *9*, *10*) agrees with our previous observations (*5*). The tumor turnover time (table 1) was calculated to be in the range of 4–7 days in a study of glioblastomas involving double radioautography. In solid tumors with a growth fraction significantly less than 1.0, the cell cycle time should be less than the tumor turnover time. In fact, we observed this in the present study.

The cell cycle time *Tc* of glioblastomas was also evaluated in tumors from patients who, after undergoing macroscopic total removal of their tumors, suffered recurrences at a later date (*16*). If there is no cell loss, the population increase can be calculated from the equation

$$Nt=N_0(1+GF)^{\frac{t}{Tc}},$$

where Nt is the number of cells at time t. If we know N_0 (number of cells at time 0), Nt (number of cells at time t), GF (growth fraction), and t, Tc can be estimated if cell loss is ignored. Nt can be estimated from the size of a tumor at recurrence by assuming 1 g tissue equals 10^9 cells. Table 3 demonstrates the estimation of Tc from the patients who underwent macroscopical total removal of their tumors and whose tumors recurred later, in the absence of additional therapy.

TABLE 3.—*Calculated cell cycle time of recurrent malignant gliomas*

Patient No.[a]	Pathology	Period of remission, days	Weight of tumor, g	Calculated cell cycle time, days[b]		
				$N_0=1$, $GF=1$	$N_0=10^6$, $GF=0.3$	$N_0=10^8$, $GF=0.3$
1	Glioblastoma	74	62	2.1	1.8	2.8
2	Glioblastoma	78	283	2.1	1.6	2.6
3	Glioblastoma	225	600	5.8	4.4	6.8
4	Malignant astrocytoma	47	128	1.3	1.1	1.7

[a] No added treatment was given after surgical macroscopic total removal of a tumor.

[b] Calculation was based on no cell loss.

If we consider only those patients who had a second operation for recurrence within 3 months (patients #1, 2, and 4), we may assume that the growth fraction is approximately 0.3 (as previously measured for similar patients). Assuming that 10^6 cells (1 mg) to 10^8 cells (100 mg tumor) remained after the first operation, calculated cell cycle times are not unlike those previously measured. Even if we assume a near total removal of the tumor (with only 1 surviving tumor cell) and a higher growth fraction (as might be anticipated with a reduction in tumor mass of this magnitude), the calculated cell cycle times also agree with our 2- to 3-day estimate obtained by direct measurements.

DRUG CONSIDERATIONS FROM A KINETIC POINT OF VIEW

Knowledge of the cell cycle time is essential in planning effective treatment with cell cycle, phase-specific chemotherapeutic agents. Drugs such as *Vinca* alkaloids, purine, and pyrimidine analogs will predominantly affect only those cells in a specific, sensitive phase of the cell cycle. Single doses of these agents may only kill cells in the sensitive cycle phase at the time of drug administration. However, if effective drug levels are maintained for a long enough period so that all cells in cycle can pass through the sensitive phase, the agent will affect all cycling cells and have its maximal effect. Unfortunately, "continuous" drug administration, either by frequent injections or by continuous infusion, for periods significantly longer than the tumor cell cycle time, may increase drug toxicity by affecting normal regenerating systems such as the bone marrow and intestinal epithelium, without increasing tumor cell-kill.

These cell cycle-specific drugs affect only a part of a population's corresponding growth fraction and, therefore, a cell-kill of 30% is the highest that could be expected with a single course of a cell cycle-specific agent in glioblastomas. This would reduce a tumor diameter at the most by about 10%. Because of the retarded dead cell removal (*17*), dead cell swelling, and proliferation of cells entering the cell cycle (*18*), much of the anticipated tumor mass reduction will not be observed. In addition, the information necessary for the exact timing of re-treatment with cell cycle-specific agents to effect a continual stepwise decrease in tumor cell population is not available now.

Obviously, treatment should include some modality that can kill nonproliferating as well as proliferating cells. The percent cell-kill would not then be limited by the growth fraction, since 100% of cells would be at risk, and a tumor cell-kill of several logs may be achieved from a single course of therapy. Drugs in this category are referred to as "cell cycle nonspecific" and include nitrosoureas, alkylating agents, and DNA binders (*19*).

To obtain a sequential decrease in tumor size with these drugs, one must repeat courses of therapy before the tumor cells have time to repopulate. The time for tumor regrowth may be calculated from knowledge of the cell cycle time, growth fraction, cell loss, and tumor cell-kill. An approximation of the minimal repopulation time for human glioblastomas may be computed if one uses a cell cycle time of 3 days, a growth fraction of 0.3, and no cell loss (since the actual rate of cell loss has not been determined). If a course of chemotherapy, either single or in combination, results in a 2 or 3 log cell-kill, it will take a minimum of 53 or 78 days, respectively, for tumor cell repopulation. In other words, even if we can reduce the tumor cell population by a factor of 0.01 or 0.001, the tumor returns to its original size in 7–11 weeks.

Furthermore, since the growth fraction increases after effective treatment (secondarily to a decrease in tumor size), the tumor repopulates more rapidly and re-treatment should be given at shorter intervals. By applying the maximal growth fraction of 1.0, we find that the minimum time for tumor regrowth is 20 or 30 days, respectively, and we may need to re-treat the patient within this 3- or 4-week interval to produce a cure. On the other hand, this period may offer the best opportunity for therapy with antimetabolites, since cycling cells predominate. If cell loss is considered in the course of tumor regrowth, the actual regrowth times might be significantly longer. In any case, the feasibility of repeated courses depends on the recovery of normal cell populations during the re-treatment interval.

It is clear that further advances in brain tumor chemotherapy will depend on obtaining more information about the pretreatment and perturbed kinetics of tumors and normal tissues.

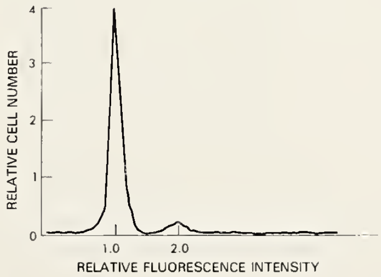
RAPID METHODS FOR ESTIMATION OF KINETIC PARAMETERS

Until recently, determinations of most kinetic parameters for the in vivo analysis of human tumors were based on laborious, time-consuming radioautographic techniques which often took 6–9 months to complete. With such methods, the patient under investigation cannot benefit from the information obtained on his tumor. Therefore, since considerable variability in kinetic parameters probably exists between tumors, it would be valuable to develop a more rapid method of determining at least some of the important factors, such as cell cycle time and growth fraction for a patient's tumor. In this manner, the information obtained may be applied directly to the patient under study and hopefully permit the planning of specific, effective therapy regimens. Two possible methods of rapid analysis presently exist.

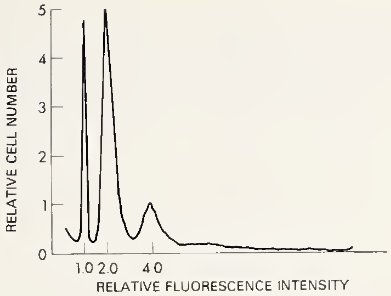
The determination of the primer-available DNA polymerase activity (PDP) index (*20, 21*) as an estimate of the growth fraction, utilizes an in vitro method that depends on the PDP. This procedure, in combination with an in vitro double-labeling radioautographic technique (*22*) to provide an estimate of the time of S-phase, permits the calculation of the cell cycle time. Both methods take about 3 weeks to complete and appear to give reliable results. Schiffer (personal communication) has investigated 2 malignant gliomas (1 glioblastoma and 1 medulloblastoma). These tumors had a cell cycle time of 2–3 days and a PDP index, which would give an estimate of 0.3 to 0.35 for the growth fraction. These results are comparable to those presented in table 2, when the previously mentioned, more time-consuming methods were followed. Another technique is the rapid, automated cytomicrofluorometric method described in (*23*). A flow microfluorometer (FMF) measures the amount of DNA per cell by shining a laser beam on the nucleus of individual cells and then measuring the intensity of the fluorescence obtained from the DNA-bound dye (acriflavin). From these data, a histogram can be constructed of the frequency with which these DNA contents appear in the cell population. This method has potential usefulness for investigating the kinetics of solid tumors.

A histogram demonstrates a single peak (number of cells vs. intensity of fluorescence) when the entire population consists of diploid cells. The sharpness of a peak indicates the relative homogeneity of the DNA contents in any one population. Multiple peaks can be found at the different positions relative to the intensity of their fluorescence, when cells with tetraploid, octaploid, etc., DNA contents exist in the population. If there are cells synthesizing DNA for duplication, they give relative fluorescence values between these peaks. However, this method has the limitation of requiring that samples be prepared in a single cell suspension. If any cell clumping exists, the FMF measures the combined DNA contents and, therefore, gives erroneous results. To date, a method for totally preventing the clumping of cells isolated from a solid tumor has not been perfected, so we have directed our attention toward developing a nuclei isolation technique for the analysis of solid tissues (*24*).

The profile illustrated in text-fig. 1 is one of those obtained from pieces of a meningioma. Profiles of other pieces from the same tumor were nearly identical in appearance. There was a large 2C DNA peak, a small 4C peak, and a few nuclei with intermediate DNA contents indicative of cells in DNA synthesis. This kind of profile suggests that this meningioma has few proliferating cells or a long cell cycle time, composed primarily of a long G_1-phase. In addition, it also suggests that this tumor is relatively homogeneous with respect to its population kinetics.



TEXT-FIGURE 1.—FMF analysis of nuclei isolated from a human meningioma. Relative fluorescence intensity values of 1.0 and 2.0 correspond to the position of nuclei with a 2C and 4C DNA content, respectively.



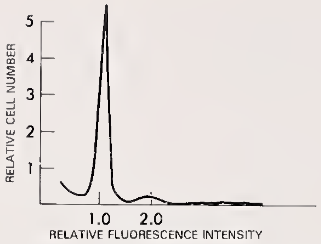
TEXT-FIGURE 2.—FMF analysis of nuclei isolated from a human glioblastoma multiforme. Relative fluorescence intensity values of 1.0, 2.0, and 4.0 correspond to the position of nuclei with a 2C, 4C, and 8C DNA content, respectively.

Text-figure 2 shows an FMF profile analyzed from a piece of a glioblastoma. This profile reveals three main peaks, a relatively sharp peak at the 2C position and a broad one at the 4C and 8C positions. Since there is a reasonable number of nuclei with DNA contents between 2C and 4C and between 4C and 8C, it is unlikely that these latter peaks are due solely to groups of 2 and 4 nuclei sticking together. In all probability, they represent the fractions of the population that are tetraploid or octaploid. The ratio of these peaks varied considerably among the samples analyzed, which indicates that this glioblastoma is much more heterogeneous with respect to its population than was the meningioma.

Tetraploid or octaploid cells are more radioresistant than diploid cells. If we see this kind of profile for a tumor, radiation would not be the therapy of choice. Of course, not all glioblastomas examined contained such prominent tetraploid and octaploid populations. However, variability was always observed when different regions of each glioblastoma were compared.

In contrast to the glioblastoma, FMF analysis of a low-grade astrocytoma showed a prominent peak in the 2C position with a small peak at the 4C position (text-fig. 3) and suggested a small proliferating population, since there were few cells between the first and second peaks. Also, it is close to the profile obtained from a meningioma. Since similar profiles were obtained from different regions of the same tumor, we must assume that this astrocytoma is a slow-growing one composed of a virtually homogeneous cell population.

The profiles A and B in text-figure 4, which were obtained from an anaplastic astrocytoma, indicate a small proliferating population as well as the existence of a tetraploid population. Histologic examination of the tumor revealed that



Text-figure 3.—FMF analysis of nuclei isolated from a human low grade astrocytoma. Relative fluorescence intensity values have the same meaning as in text-figure 1.

sections A and B consisted primarily of well-differentiated astrocytes with microcysts, whereas sections C and D consisted of pleomorphic and/or giant cells. Interpretation of such a small number of human tumors is difficult, but it should be obvious that this type of information is invaluable in the design of therapeutic protocols. In particular, the rapidity of this method could allow a patient's postsurgical treatment to be based on the kinetic information obtained from his tumor.

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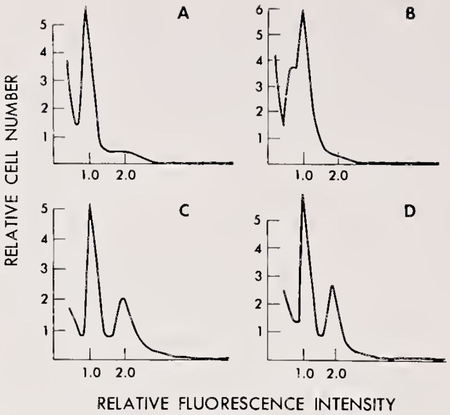

Text-figure 4.—FMF analysis of nuclei isolated from a human anaplastic astrocytoma. A, B, C, and D represent profiles obtained from four regions of the same tumor. Relative fluorescence intensity values have the same meaning as in text-figure 1.

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DISCUSSION I

K. Charyulu: Would Dr. Blasberg elaborate some of the techniques for altering the blood–brain barrier, whether for chemotherapy or another approach, such as immunotherapy?

R. Blasberg: The whole matter is highly experimental. The pharmaceutical chemist may be able to alter the physical characteristics of a particular chemotherapeutic agent, e.g., to increase the penetration of a more lipophilic drug into cerebral capillaries so that it will achieve a higher extraction fraction in tumor and adjacent tissue. The situation is complex and the data I presented were oversimplified. When you are dealing with a neoplasm of the CNS, you do not have an intact blood–brain barrier in most cases; there is a gradation so that the capillaries are not all "tight," that is, impermeable.

C. Wilson: You tease us a bit by saying that you are not doing it with drugs, but preliminary treatment with hyperosmolar agents produces this effect of enhanced permeability.

Blasberg: Yes. Stanley Rappaport described this, and we are working with him to see whether this effect can be applied to the treatment of CNS disease. Other things will do it, too. Igor Clotso and Stanley Rappaport showed that an episode of hypertension, i.e., a bolus of drug or a rapid infusion of saline, will open the capillaries transiently. There are many ways to do this.

Wilson: A simple way to do it is by cerebral angiography, which affects the permeability of the normal capillary endothelium.

A. Freeman: Dr. Wilson, you showed in your experimental tumors that methotrexate, in what is now regarded as low levels, increased permeability. Have you tried methotrexate in high levels in your experimental model system?

Wilson: Are you referring to the systemic administration of methotrexate?

Freeman: Yes.

Wilson: Most of these studies use graded doses so that some of them are exceedingly toxic; yet, even at those levels, they saw no activity. For a tumor located in the CNS, the effect of systemically administered methotrexate is so small that it is not worth the toxicity. Some studies have been done with high-dose methotrexate, given intrathecally, with subsequent rescue, but no one has had anything encouraging to say about systemic methotrexate, even long-term infusions.

A. Evans: I understand that doctors at Memorial Sloan-Kettering are using high-dose methotrexate as a drug of choice for CNS tumors.

A. Bleyer: Drs. Shapiro and Rosen at Memorial found responses in 4 of 8 patients with brain tumors who were treated with high-dose systemic methotrexate. Dr. Trossy stated that 6 of 7 of his patients responded to high-dose methotrexate (in the range of 1,000–2,200 mg/kg over a short infusion).

When thymidine is given iv, do its physicochemical kinetics permit sufficient uptake by tumor cells so that it can be used to quantify labeling indices and growth fractions? That is, are these cells exposed to thymidine in the extracellular fluid after iv administration? The accuracy and interpretation of the data you have shown depend, in large part, on that.

Wilson: You are saying that if the thymidine cannot get there, then what we seem to be seeing is not true. Well if thymidine is not absorbed there, nothing else, such as glucose, is apt to be either. Therefore, it is unlikely that thymidine is going to miss any metabolically active cells.

T. Hoshino: If you examine the slide of such a heterogenic tumor, you will find that some areas of the tissue that had been treated with a pulse of tritiated thymidine are not labeled. I disregard those places because I could not clarify whether they had been perfused adequately by the pulse of thymidine, or whether they had been in cell cycle at the time the pulse was administered. The areas where a reasonable number of labeled cells were distributed were taken into account, since all of the surrounding cells had equal opportunity to take up thymidine. I do not know if there is a critical time barrier for thymidine to penetrate tumor cells by means of shunting or occlusion of feeding vessels.

Wilson: This brings up the subject of sanctuaries within a tumor, e.g., an anatomical sanctuary, which perhaps thymidine cannot penetrate. Also, maybe the nutrients that the cells need fail to reach there. How grim can things get for a glial cell and have it still survive? Then as the situation improves, when will it again become a clonogenic cell? We do not know.

Certainly, serious diffusion problems can arise during in vitro studies with thymidine. In his work, Dr. Hoshino has been careful to avoid the diffusion problem, but I do not know how it can be avoided in vivo. I was most interested in the comments concerning high-dose systemic methotrexate. I am much impressed by any form of treatment that benefits 6 of 7 patients with a primary brain tumor.

J. Swenberg: I do not think I would dismiss those areas Dr. Hoshino referred to because, when we have given thymidine, we also found areas of unlabeled healthy cells adjacent to the heavily labeled areas. I think these are nests of cells that are turning over and others that are not. I would not dismiss unlabeled areas, because calculations that exclude them will probably be in error.

Wilson: Are these just nonproliferating cells?

Swenberg: Yes.

Wilson: They are certainly viable.

Hoshino: Yes, it is possible. Still it is hard to demonstrate whether these cells *could* not get tritiated thymidine or they *did* not, when we saw the nest of unlabeled cells. This certainly biases calculation, but at least we can say the results are obtained from the areas that had been perfused by the pulse of tritiated thymidine.

Blasberg: There is a simple way to do that. Inject some thymidine and sample the tissue right away. What you are looking at several hours after is residual or incorporated thymidine, not free thymidine. You will have to inject some in an animal and see if it gets into the brain.

Wilson: It has been done with drugs but to get the data we require, it would have to be done with something with characteristics similar to those of thymidine.

L. Rubinstein: I have two questions for Dr. Hoshino. The first regards the labeling index in his tumors: I assume that his figures take into account the differential uptake between the tumor cells and the vascular endothelial proliferation, which is marked. Second, does he have any data concerning differences in the growth fraction and cell cycle time between the original tumor and the glioblastoma and subsequent recurrence(s) of the tumor?

Hoshino: Concerning the second question, I studied only recurrent glioblastoma patients. I do not know if there are any differences in growth fraction and cell cycle time between the primary and subsequent recurrence of the tumor.

I believe the glioblastomas have quite characteristic patterns of vascular proliferations. Generally, these vessels in the tumor proliferate to support its growth and are *not* neoplastic cells. However, in glioblastoma I often observed the heavier labeling in those endothelial or adventitial cells than in the surrounding tumor cells. On the contrary, the endothelial cells of the vessels in metastatic brain tumor revealed a lower labeling index than did the surrounding tumor cells. It seems to me that vascular components in glioblastoma may have some capacity to proliferate independently from surrounding tumor cells, as if they also are neoplastic.

Wilson: Dr. Rubinstein's question to Dr. Hoshino is whether, when calculating his labeling indices, he includes all cells in the field and the endothelium, or is he speaking only of those cells that are definitely glial? If he ignores the labeling in vessels, that would really be striking.

Rubinstein: Does he ignore such labeling?

Wilson: Yes. It is interesting that in one well-known model in which one can study both vessels and tumor cells, the vessels proliferate at the same rate as the cells, but the vessels cannot keep up because they have about twice as long a cell cycle time. Thus no matter how hard they work, they cannot keep up, which explains the necrotic areas in this particular tumor as it became larger.

A. Fleischer: I have a question for Dr. Blasberg, particularly. I am especially interested in intra-arterial infusion of chemotherapeutic agents, in view of the several advantages to this route of administration. Have you any data concerning chemotherapy by this route, and do you know any model that demonstrates increased embedding in tumor?

Furthermore, I think you said that when it was administered intra-arterially, delivery of the drug to tumor was higher if the cerebral blood flow was lower. Would you elaborate?

Blasberg: The point about the blood flow was discussed at a recent conference in New York. With respect to specific studies concerning the treatment of solid tumors with intra-arterial drugs, most of these have been done in head and neck cancer. I tried to outline certain criteria that the clinician should take into account if he is going to choose that route of administration. These criteria are useful, but I am not sure they are being met. Intra-arterial infusions for solid tumors have frequently failed, partly because certain of these criteria have been ignored.

Wilson: Dr. Victor Levin from our laboratory has had a paper accepted for publication (I think in *Cancer Treatment Reports*[1]) dealing with intra-arterial BCNU. Is there an advantage to intra-arterial BCNU? Yes, because it avoids systemic toxicity. Once you attain about an LD10, you have a 3-log kill and no more cell-kill, no matter how much BCNU you give. The drug effect is self-limiting at 3 logs. However, you can get that dose of BCNU in with a smaller systemic burden by giving it intra-arterially. The factor, as I recall, is about 2.5 to 3; in other words, an intra-arterial dose that much smaller gives you the same cell-kill as you would get systemically. You would use it only in special situations, i.e., when the patient had a compromised bone marrow but was still responsive to BCNU.

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G. D'Angio: I would like to sound a note of caution concerning high-dose methotrexate because it seems to be a radiation enhancer. When I was at Memorial Sloan-Kettering Hospital, I saw patients who seemed to have sustained brain damage that was probably attributable to the combination of high doses of radiation followed by high doses of methotrexate. Anybody embarking on this type of program should contact Dr. Rosen at Memorial concerning his experience before trying it on patients who have had prior irradiation.

Wilson: Thank you. I must confess my prejudice concerning methotrexate is based on simple kinetic considerations. At most, only about 10% of the cells are affected. Our group has decided not to pursue this particular cell cycle-specific agent; although, as Dr. Hoshino pointed out, it would be valuable to have an agent to insert between pulses of a highly effective drug like BCNU that is cell cycle nonspecific but cannot be given frequently.

P. Paoletti: I would like to present briefly the results obtained with BCNU, CCNU, MeCCNU, and GCNU, centrally administered to rats at the natals area for cerebral tumors induced by transplant. The tumor model used has previously been described. Ten milligrams ENU/kg was administered in a single iv dose to pregnant rats after 16 days of gestation. Eighty-nine percent of the offspring developed tumors of the nervous system within 400 days of birth. Two different experiments were performed on the newborn rats.

Experiment 1 consisted of the administration of 10 mg BCNU/kg ip to 19 animals; CCNU, 20 mg/kg orally to 19 animals; and 10 mg 3-(tetra-acetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea (GCNU)/kg ip to 21 animals. The injections were started at the 3d month after birth and were given every 6 weeks for 4 administrations. Fourteen animals served as controls.

In experiment 2, 5 mg BCNU/kg was administered ip to 23 animals; 35 mg CCNU/kg orally to 14 animals; and 35 mg MeCCNU/kg orally to 17 animals. Each drug was started at the 2d month after birth and given every 4 weeks for 4 administrations. Fourteen animals were kept as controls. In the first experiment, groups of treated and untreated animals were killed at the 6th, 7th, 9th, and 14th month after birth, and in the second experiment at the 6th, 7th, 8th, and 9th month.

In experiment 1, 71% of the control rats developed tumors of the nervous system. The number of tumors was greatly reduced by BCNU and CCNU, whereas GCNU appeared to be much less effective. The in vitro effect is more evident for CCNU than for BCNU at the 6th, 7th, and 9th month than at the 14th month after birth. The number of multiple tumors is also strongly reduced, particularly by CCNU.

In experiment 1 with BCNU, the histologic pattern of the tumors was unmodified in comparison with 8 of the controls, whereas the CCNU neurinomas or polymorphic gliomas were not observed. All the other endogliomas were small and were poor in mitosis. The absence of neurinomas in the CCNU-treated animals was remarkable. On the contrary, polymorphic gliomas developed with GCNU. Also, no neurinomas were seen with GCNU. Only endoglial micro tumors were observed after GCNU was administered, but not after BCNU or CCNU were given.

In experiment 2, 86% of the control rats developed tumors of the nervous system. The animals were followed only until the 9th month after birth. After this point, they began to die from extratumoral causes. The immediate effect of BCNU and CCNU is less evident than in the first experiment, but the total number of induced tumors is clearly lower. MeCCNU gives better results than CCNU. The number of multiple tumors that developed after BCNU and CCNU were given is higher than in the first experiment but lower in comparison with control animals.

The histologic pattern is different from that described in the first experiment. There is a remarkable development of neurinomas after BCNU and CCNU but not after MeCCNU.

In summary, synthesized BCNU, CCNU, and MeCCNU are effective in preventing the formation of tumor after injection into the natals area of pregnant rats.

Wilson: Dr. Donald Slagel has two questions directed to Dr. Blasberg. First, would he compare the ultrastructures of intercellular junctions of ependyma and capillary endothelium? Secondly, Dr. Slagel asked about the drug binding of proteins normally present in CSF.

Blasberg: First, the ependymal cells do not have tight junctions; they are open. Not only do drugs or small extracellular marker compounds, such as EDTA or sucrose, diffuse from the CSF into the ECF of the brain, but large molecules, such as peroxidase, also do so, as was clearly demonstrated by Milton Brightman. We have even observed albumin moving across the endothelial or, rather, the ependymal cells.

Now a special area of ependymal cells (if you wish to call them that) or overt choroid plexus does have tight junctions. That is where the "blood–CSF barrier" is thought to exist, at least on an ultrastructural basis. The endothelial cells in the choroid plexus are open, in contrast to the endothelial cells of brain capillaries.

Wilson: Now the second question, the binding to CSF protein.

Blasberg: This area has not been investigated or even really thought out; certainly, the serum proteins act, or can act, as a significant reservoir for some drugs. In some cases up to 90 or 99% of drug is bound to serum proteins. However, no one has yet explored the effect of this reservoir of protein–drug penetration into the tumor and adjacent brain. Certainly, the reservoir effect will be much less.

P. Gutin: I wish to contradict Dr. Blasberg's delivery scheme. At the Baltimore Cancer Research Center we have found thio-TEPA, an extremely lipid-soluble drug, to be an attractive addition to the intrathecal drugs used for meningeal tumors. Do you think your pharmokinetic delivery schema applies to meningeal tumors as well as deeper parenchymal ones?

Blasberg: It is difficult to say because we are dealing with a complex of factors. I can envision that thio-TEPA, administered into the lumbar sac, would be quite effective in that immediate region, i.e., if the course of action of the drug is relatively short before it has had time to diffuse into the brain and through brain capillaries after the systemic circulation. Yes, it should confer a considerable advantage.

If you are going to treat meningeal disease over the cerebral hemispheres by administering drug into the lumbar sac, I would raise certain serious questions. The transit time is in the order of hours, and I am not sure that thio-TEPA will remain active over that period of time. If it is active over the cerebral hemispheres, I suggest that the drug has diffused through lumbar capillaries and has recirculated through the systemic circulation and is now effective as a systemic agent.

M. Walker: I believe intrathecal thio-TEPA needs to be placed in perspective. Dr. Blasberg's data showed clearly that, intrathecally, this drug should be leaving the CSF by every conceivable route within a few minutes. I think that he is correct and his experimental data are correct, but it is profoundly disturbing to accept the fact that this drug is leaving so rapidly.

As another intrathecal drug, I think it is of modest value in patients who have been treated with several other drugs intrathecally, but it is one we can study and can use now. Perhaps we are not using the correct dose–timing relationships with thio-TEPA; e.g., multiple small doses intrathecally might be better because the drug would persist in the CSF a good deal longer than does a single higher dose. However, this scheduling has not been tried, so we do not know the answers yet.

The highest dose of thio-TEPA reached in the phase I study was 12–15 mg thio-TEPA per m^2, and that is an unbelievably high dose to place intrathecally. It is almost the same amount as is given systemically, which is a tremendous amount of drug. We may be overwhelming the entire kinetics and driving the drug at that particular point.

H. Abelson: Concerning methotrexate, I want to reflect the skepticism of Dr. Wilson and underline the note of caution sounded by Dr. D'Angio. Several years ago, Dr. Bertino showed that normal brain contains no dihydrofolate reductase, which classically has been thought to be the enzyme through which methotrexate exerts its cytotoxic effect. So if we want to be rational about methotrexate for brain tumors, we must establish that these tumors contain that enzyme, since it is apparent that normal brain does not. Some preliminary evidence suggests that glioblastoma multiforme does not contain this enzyme. We must proceed cautiously, particularly in view of the tremendous amount of toxicity that has been seen with (albeit some people say is an effect of) high-dose methotrexate.

C. West: I would like to make a few observations about procarbazine in relationship to protein binding in the CSF. First, the form of this drug is controlled by the pH. In plasma at pH 7.4, 85% of it is un-ionized. Also, it is highly lipid soluble, at least in chloroform, in which it has a greater than 1:1 distribution.

If administered orally to monkeys, the plasma level is only half that reached by iv or carotid administration. It is not bound in CSF; however, more than 50% of it is bound in the plasma during the first 3 hours. Thereafter, when the plasma level drops low, it is highly bound at that level. It is excreted primarily in the urine, at least the N-methyl moiety is excreted partly by the respiratory tract and partly by the urine; the ring part of the drug is excreted primarily in the urine.

W. Bingham: One of the outstanding characteristics of the malignant gliomas is arteriovenous shunting. We see this angiographically and also at the operating table. In view of this well-documented and commonly observed phenomenon, why are we considering intra-arterial infusions?

Wilson: Thank you for the question. Intra-arterial chemotherapy for brain tumors ignores other important facts. For example, few of these tumors are within the distribution of one carotid artery; for instance, the type of vessel that one sees within a glioblastoma. We also ignore the AV shunts and the problems of delivering the drug to the tumor. Our facts seem to mediate against intra-arterial therapy for a malignant glioma.

A. Freeman: Dr. Swenberg, you have shown us the viruses of induced tumors in the lower animals. Is there any evidence of footprints of a viral oncogene in the human gliomas?

Swenberg: There is no such evidence. The marmoset, which is a primate, is the closest we have come to man.

Wilson: Does Dr. Yung not have some provocative evidence of virus (MuSV) in some human gliomas in culture?

Rubinstein: Some provocative new work refers, not to the gliomas, but to the meningiomas. Yung's group reported that a large proportion of meningiomas have a specific chromosome defect (absence of the 22 chromosome), and they also published immunofluorescence pictures of some of these chromosomal abnormal meningioma in culture, showing immunofluorescence against the SV40. This work, of course, is puzzling and needs to be confirmed.

Swenberg: May I make a few comments regarding our ASV chemotherapy model? First of all, we did not get an effect on the procarbazine trial; however, we also did not get to toxic doses; I think that the case is still open.

In Dr. Victor Levin's experience and in our own, another drug, PCNU, looks promising. Recently, it was approved for large animal toxicology trials, and we hope it will be available for advanced study in the near future. In our hands and in Dr. Levin's, this particular drug seems to be as good as BCNU if not better.

I should emphasize that these compounds should always be studied for other effects. The Russians are using MNU, and a paper in *Cancer Chemotherapy Reports* (now known as *Cancer Treatment Reports*) suggests that MNU, ENU, and the higher analogs are effective against tumors. In fact, they have accumulated considerable data in man. With each new drug, the investigator must seek to compare the relative carcinogenicity versus the efficacy in therapy. These compounds are extremely potent; procarbazine, for example, is a potent carcinogen when administered transplacentally in rats.

Wilson: I was not fully aware of this. The ASV model is attractive for many reasons, yet if it missed procarbazine it would be less so. If we go back and use higher doses, the model may pick up procarbazine.

M. Lane: With respect to earlier comments regarding intrathecal therapy with methotrexate and the need for folate reductase, we should keep an open mind on this aspect of brain tumor therapy. Several basic biochemical papers have suggested that dihydrofolate reductase may not be the primary site of action of methotrexate, and that, indeed, this may act at the level of the thymidylate synthetase enzyme.

Wilson: I should say that my aversion is acquired, not inherited or congenital. Ten years ago, Dr. William Newton (Columbus, Ohio) and I were administering as much methotrexate intrathecally as anyone I knew. However, I would not want you to leave thinking that I believe methotrexate is useless in the CNS. Quite the contrary. Times change, and we have to decide what course of action will be the most productive.

Correlation of Animal Brain Tumor Models With Human Neuro-Oncology[1,2]

L. J. Rubinstein[3]

ABSTRACT—This paper reviews the histopathology and cytopathology of a number of experimental animal brain tumor models to determine their relevance to human neuro-oncology. The models discussed include brain tumors produced by local implants of carcinogenic hydrocarbons and by the administration of resorptive N-nitroso compounds, some of the cell lines derived from the latter, tumors resulting from the intracerebral inoculation of oncogenic viruses, and the differentiating neuroepithelial component of a transplantable mouse teratoma. The structural and biochemical criteria that can be applied for the identification of the various stages of neuronal and glial cell maturation are highly precise. They reveal a wide diversity of tumor cell types that are now available in experimental neuro-oncogenesis.—Natl Cancer Inst Monogr 46: 43–49, 1977.

This article will examine a number of animal brain tumor models in the context of their histologic equivalence to some of the recognized nervous system tumors encountered in man. Although many other factors must be taken into account before a biologic correlation can be established, a demonstration of histologic equivalence between experimentally induced and human neural tumors would do much to establish the validity of the various animal models to the clinical situation. Many such models have been proposed, but this review will examine only those that have been used most frequently.

MODELS RESULTING FROM LOCAL IMPLANTS OF CARCINOGENIC HYDROCARBONS

The most publicized model of this kind is a transplantable, malignant, primitive, neuroepithelial murine tumor induced by the intracerebral implantation of a 3-methylcholanthrene pellet (*1*) and maintained in several laboratories by serial subcutaneous passage (*2–7*). It has been used extensively in experimental situations, e.g., for its response to single doses of irradiation after subcutaneous implantation (*2*); its response to fractionated doses of brain irradiation after intracerebral implantation (*4*); to study the inhibiting effect of prior intradermal immunization with a tumor–adjuvant mixture (*3*); for the fine structural study of peritumoral cerebral edema in mice (*8*); and, more recently, for its response to the application of various chemotherapeutic agents, including 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) (*9*), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) (*10, 11*), vincristine, 5-fluorouracil (*11*), cyclophosphamide, mithramycin, and methotrexate (*9*), after cerebral implantation.

From the histologic point of view, the exact nature of this neoplasm is difficult to determine because of its poorly differentiated cellular composition. By light microscopy, it consists of cuboidal or polyhedral cells often arranged in distinct cords separated by a scant supporting stroma (*3, 4, 6, 7*). Although widely designated as an "ependymoblastoma," electron microscopy does not demonstrate ependymal differentiation (*5*) and does not suggest that the tumor cells are at any time determined to develop into mature ependymal cells [*see* (*12*), for discussion]. It does not correspond to the rare human ependymoblastoma (*13–15*), a primitive neoplasm in children, in which ependymal differentiation is clearly recognized. Its relationship to any human equivalent is obscure, a fact that limits its validity in the context of human nervous system neoplasms. This mouse tumor expresses large quantities of viral particle aggregates that have been identified as murine mammary tumor virus (*7*).

NEUROGENIC TUMORS INDUCED BY RESORPTIVE N-NITROSO COMPOUNDS

N-nitroso compounds are widely used to produce neoplasms of the central and peripheral nervous systems (CNS, PNS). They yield a large variety of neurogenic tumors with constant regularity depending on the compound selected, the animal species, the strain, and the method of administration. Experimental animals of choice are various strains of the rat. The tumors most often studied until now have been those produced

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by the repeated iv injection of methylnitrosourea (MNU) in young adult animals or, more conveniently, from a single, low iv dose of ethylnitrosourea (ENU) in the 15th- or 16th-day pregnant dam, which results in almost a 100% incidence of neurogenic tumors in the offspring. The recent literature (*16–25*) describes many contributions made possible through variants of this model.

The tumors in the PNS most frequently obtained are schwannomas or neurinomas of various degrees of malignancy depending on the compound used, the technique of administration, and the animal strain. Usually, the more anaplastic tumors are obtained following transplacental ENU in Sprague-Dawley or Long-Evans rats (*17, 26, 27*), whereas repeated iv injection of MNU in Fischer rats produces relatively more differentiated schwannomas (*21, 24*). The most frequent site of tumorigenesis is the trigeminal nerve root (*16, 17, 20, 23*), followed by the spinal nerve roots. Although it seems to be clearly established that these tumors originate from Schwann cells, even when they are most anaplastic (*17, 25–27*), significant biologic differences exist between experimental neurinomas in the rat and spontaneous tumors in man respecting their grades of malignancy and preferential localizations. For example, malignant change in human schwannomas is exceptional, and tumors of the trigeminal ganglion and nerve root are rare in man.

The tumors in the CNS in this model are virtually all gliomas, and neuronal tumors are seldom obtained [*see* (*28*) for an exceptional example]. The subependymal cells of the cerebral hemispheres appear to be especially susceptible to the action of the resorptive carcinogen. Almost certainly, the target cells are the still-cycling spongioblasts or glioblasts caught in the course of their migration from the primitive ventricular zone [*see* (*29*) for discussion]. Both polycentric gliomas and gliomas of multiple cell types are frequent, and this results in a mixed neoplastic population in which the different cellular components cannot always be identified with certainty.

On the other hand, despite the apparent vulnerability of the still proliferating cells of the fetal external granular layer of the cerebellum to the cytotoxic action of N-nitroso compounds (*29*), this model does not produce cerebellar medulloblastomas, apart from exceptional instances reported in an occasional rat (*30*) and in a few mice (*31*). Furthermore, these animal gliomas usually do not show the classical histologic picture of glioblastoma, with which the pathologist is familiar in man.

The most frequent type of pure glioma produced in this model is the oligodendroglioma, although good examples of astrocytoma may also be obtained (*32*).[4]

TUMOR CELL LINES DERIVED FROM N-NITROSO-INDUCED RAT GLIOMAS

One of the most extensively characterized of these lines is the C-6 glioma, a line of cells cloned from an MNU-induced rat glioma (*18, 33*) that has been widely used for the study of the composition and function of glia. This cell line has been investigated in several laboratories for the presence of specific surface antigens, its response to antimetabolites and vitamin B_{12} depletion, the production of complex carbohydrate compounds, the presence of various enzymes implicated in active transport and cyclic AMP metabolism, its behavior in organ culture systems [*see* (*34*) for references], and its potential in cell hybridization experiments (*35, 36*). Morphologically, the line represents a still poorly differentiated glial cell type that has the potential to differentiate into stellate astrocytes, i.e., a potential which is expressed in an in vitro system that favors differentiation by an increased production of glial fibrillary acidic (GFA) protein, a specific marker for fibrillary astrocytes (*34*). Such an increase, originally demonstrated qualitatively by specific immunofluorescence against GFA protein antiserum, later was confirmed by a quantitative immunoradiometric assay, which showed increases of GFA protein ranging from a factor of 7 to 40 when the line is maintained in an enriched medium in the appropriate culture system (*37*). This increased production of a specific antigenic determinant occurred in the absence of gliofibrillogenesis in the in vitro system used, which suggested that the expression of specific cellular products may not coincide with structural astrocytic differentiation.

Another cell line, also initially obtained from an MNU-induced rat glioma (*18, 19*), has been used extensively as an animal brain tumor model in another series of experiments, e.g., for its response after cerebral implantation to treatment with BCNU (*38*) and for the study of its 1) proliferation kinetics (*38, 39*), 2) behavior in cell culture (*40*), 3) in vitro response to BCNU (*41*), 4) colony-forming capacity in vitro after BCNU chemotherapy in vivo (*42*), and 5) cellular radiosensitivity to single doses of ionizing radiation (*43*). To the histologist this line, now designated as 9L (*41*), presents a problem because reports state that after serial animal and cell culture passage, the original tumor changed from a typical malignant astrocytoma to a reticulin-forming neoplasm identified as either sarcoma or gliosarcoma (*38*, *42*). In the light of accepted histopathologic experience the hypothesis that typical glioma cells may convert into sarcoma is difficult to entertain, but we know that mixed gliomas and sarcomas occur in man (*44*). Their identification is usually easy. Their development is most frequently explained on the assumption that the cellular stromal elements (either endothelial or fibroblastic) that accompany a malignant glioma have undergone coincidental or consecutive sarcomatous transformation. Although it is possible that the rat glioma may have undergone such an evolution in the course of serial passage, the sequence of morphologic events is somewhat unclear because no one has identified unequivocal transitional stages in this model. Greene and Harvey (*45*) have reported that hamsters and mice develop independent sarcomas following the subcutaneous transplantation of guinea pig brain tumors (derived from human glioblastomas) and their adjacent hyperplastic vascular stroma. However, those sarcomas arose in the subcutaneous tissues of the recipient animals, not in the brain. The possibility should be raised that either in vivo or in vitro transformation of the accompanying fibroblasts may have occurred in the course of transfer in the model under discussion, because spontaneous transformation is a well-established phenomenon in continuous cell culture (*46*). This is almost predictable in murine cell lines of all laboratory strains tested, and has been documented also in other animal cell lines, including rat fibroblasts (*47, 48*). At this stage, it is probably impossible to determine the origin of the sarcoma cells in the 9L cell line. If it were possible to examine this model afresh, we perhaps could establish the origin of the transformed fibroblasts by tissue histocompatibility typing in the case of allogeneic transplantation, or by male–female chromosomal analysis in the case of syngeneic transplantation. An additional complication, of course, is that the virogene theory, applicable to the various laboratory mouse strains, may be applicable to the laboratory rat also.

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[4] In the above paper (*32*), the strain of rats used was incorrectly designated as Long-Evans instead of Fischer (Erratum in *Lab Invest* 32:778, 1975).

NEURAL TUMORS OBTAINED WITH ONCOGENIC VIRUSES

Among the RNA tumor viruses, the avian sarcoma virus (ASV) has been used most successfully for the production, by the intracranial route, of nervous system tumors both of glial and sarcomatous nature. The yield is high and rapid, amounting to 100% in animals inoculated soon after birth. The characteristics of this group of tumors have been described in several recent studies (*49–53*). From the morphologic point of view, the model has considerable relevance to human tumors. The gliomas obtained in dogs after the neonatal intracerebral injection of the Schmidt-Ruppin strain of Rous sarcoma virus (RSV) are virtually identical with the pilocytic astrocytomas of juvenile type encountered in man (*50*). As with gliomas induced with resorptive alkylating agents, the tumors often tend to favor the region of the subependymal plate in the cerebral hemispheres (*49, 50*). Unlike the gliomas induced with N-nitroso compounds, the most frequent glial neoplasms in the rat are astrocytomas (*51, 53*). Those obtained following the inoculation of the Bratislava 77 strain of RSV in Fischer rats are cytologically indistinguishable from the various grades of cerebral astrocytoma commonly encountered in man (*53*).

Among the neural tumors obtained with DNA tumor viruses, an interesting model results from the inoculation of the human adenovirus type 12 (*54–58*). Although these tumors have been labeled embryonic gliomas in hamsters (*54*) and medulloepitheliomas in Sprague-Dawley rats (*55*), the illustrations and descriptions suggest that they are ganglionic neoplasms. When the virus is introduced intracerebrally, the neoplastic transformation is expressed as neuroblastoma (*54, 55*); when it is injected intraocularly, retinoblastomas are obtained (*56, 57*); and when the virus is injected ip, neuroblastomas again are produced (*58*). In this model the viral target therefore appears to be exclusively neuronal. In addition, unlike almost all the experimentally induced neural tumors produced by resorptive alkylating agents or by oncogenic viruses (which are comparable to human gliomas of adult cell types), those obtained with the human adenovirus type 12 have the features of an embryonal neuroepithelial neoplasm.

In regard to the papova group of viruses, it is likely that most of the intracerebral tumors obtained after the local injection of SV40, which initially were thought to be ependymomas, are choroid plexus papillomas or carcinomas (*59*). The JC strain of the papovavirus, which causes human progressive multifocal leukoencephalopathy (PML), can induce malignant brain tumors in a high percentage of hamsters following its intracerebral or sc inoculation at birth (*60, 61*). The most frequent of these tumors is the cerebellar medulloblastoma, probably arising from the internal granular layer (*61*). This experience raises the hope that a reproducible animal model for the human medulloblastoma may at last be available.

MOUSE NEUROBLASTOMA CELL LINES

The C-1300 mouse neuroblastoma cell line is used extensively in several laboratories for the study of morphologic (*62, 63*) and biochemical (*62, 64*) neuronal differentiation, for the production of enzymes necessary for the synthesis and degradation of neurotransmitter substances, for its ability to generate action potentials (*65, 66*), and for cell hybridization experiments in conjunction with L-strain fibroblasts (*67, 68*) and cloned glioma cell lines (*36, 69*). Several experiments suggest that these neuroblastoma cells can differentiate in vitro and develop neuritic extensions, especially when exposed to putative inducers such as cyclic AMP (*70*) or prostaglandin (*71*). However, differentiation seems to stop short of full ganglionic maturation as evidenced by the failure so far to induce synapse formation.

MOUSE TRANSPLANTABLE TERATOMA MODELS WITH DIVERGENT NEUROEPITHELIAL CELL POPULATIONS

Most of the established animal brain tumors could serve as models for human gliomas composed of mature cell types, with the exception of those obtained with the human adenovirus type 12 and with the JC strain of human papovavirus. A possible model for the study of divergent neuroepithelial differentiation in embryonal tumors of the CNS in man, a group of neoplasms that selects children and is especially complex in its identification and classification (*72*), is a transplantable mouse teratoma, of which various cell lines are available (*73–76*). These experimental tumors can be produced by the grafting of pregastrulation embryos into the testis or beneath the renal capsule of susceptible inbred strains of adult mice. The teratomas are multipotential and malignant. The progeny of the primitive stem cells of these tumors, which can be either in ascitic or solid tumor implant form, differentiates into derivatives of the three classical germ layers.

The solid tumors derived from the OTT-6050 cell line, originally developed by Stevens (*73*), contain a high percentage of neuroepithelial cells, estimated from serial section of one entire transplant processed in our laboratory to 80–90% of the cell population (*75*) in some cases. Study of this model by light (*75*) and electron microscopy (*12*) has permitted a detailed morphologic analysis of the various stages of neuroepithelial differentiation and has provided valid histologic equivalences both to the stages of cytogenesis that have been described in the developing avian and mammalian CNS and to a number of known types of embryonal tumors in the CNS encountered in man. These stages range from areas of still undifferentiated but multipotential medullary epithelium, mimicking the primitive neural tube and corresponding to the cerebral medulloepithelioma, to neuroblastic and ganglionic areas on the one hand (corresponding, respectively, to neuroblastoma, ganglioneuroma, and ganglioglioma) and to divergent stages of glial differentiation on the other (corresponding to astrocytoma, oligodendroglioma, ependymoblastoma, and ependymoma). Similar light and electron microscopic observations have been made on other mouse teratoma models (*74, 76*). The precise morphologic criteria that determine the various cell types in divergent neurocytogenesis have been successfully applied in these studies, permitting, for example, the establishment of clear distinctions between primitive medullary, ependymoblastomatous, and ependymal·rosettes (*12*). Ganglionic maturation is unequivocally demonstrated by the identification of bundles of neurites and by the tracing of axonal processes to the cytoplasm of neuroblasts with the use of specific silver impregnations (*75*), and by the presence of dense-core vesicles (*12, 76*) and of well-formed synaptic complexes (*12, 74, 76*) by electron microscopy. Biochemically, cyclic AMP, serotonin, and enzymes involved in the metabolism of biogenic amines and acetylcholine are demonstrable (*77*). Indirect immunofluorescence with antisera to the GFA protein also has been applied to this model, both in vivo (*75*) and in vitro (*78*), to confirm astrocytic differentiation.

The potential significance of the mouse teratoma model extends well beyond its narrow relevance to the study of embryonal tumors of the CNS. Today, increasing attention is being paid to the concept that neoplasia could be the pathologic counterpart of normal differentiation or, more precisely, the phenotypic expression of an aberration in the normal programming of gene activity induced by epigenetic mechanisms (*79–83*). In neuro-oncology, this concept has been illustrated by the experiments of Artzt and Bennett (*84*) on mice bearing a specific allele that causes defective primitive streak formation and its subsequent overgrowth in homozygous animals (t^{w18}). Implantation of these genetically aberrant embryos into the testes of adult mice results in an appreciable incidence, in the recipients, of tumors comprising large areas of embryonal neuroepithelial tissue that appear equivalent to the medulloepithelioma, the medulloblastoma, and the neuroblastoma in man. However, the transplantability of these tumors is limited, and after one passage, they develop into well-differentiated teratomas that can no longer be transplanted. Thus a specific genetic factor that normally codes for a block in early development can be made to express an error of differentiation in the form of a largely embryonal tumor of neural type.

So far, such a model has not produced a pure neoplasm composed entirely of neuroepithelial cells, but Damjanov, Solter, and Serman (*74*) have described a transplantable mouse teratoma composed partly of embryonal undifferentiated stem cells, in which the capacity for differentiation is apparently restricted solely to neuroepithelial derivatives. This promising line of research is being actively pursued in a number of laboratories at this time. There clearly are differences between the various mouse teratoma models. These differences are related to some of their biologic properties, in particular their capacity to metastasize, susceptibility to demonstrate orderly progressive differentiation, and ability to develop certain components of the CNS, such as myelin and microglia.

In conclusion, the precise structural antigenic and biochemical criteria applicable to the identification of mature ganglionic and glial cells and to the various stages of normal neuronal and glial cytogenesis are also applicable to the different animal tumor models used in experimental neurooncology. These criteria reveal a wide diversity of tumor cell types. Furthermore, there are significant morphologic and biologic differences as well as similarities between the various laboratory models and naturally occurring human nervous system tumors. For this reason, each model deserves critical evaluation if we are to draw valid inferences from the experimental data and apply them to human cancer therapy.

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Nomenclature for Gliomas[1]

F. Stephen Vogel[2]

ABSTRACT—Gliomas, derived from astrocytes, oligodendroglia, or ependyma, are each united into a continuum by a graduation of anaplasia. Neoplasms originate at all levels of each continuum; subsequently, some move along its declivity. Conversely, neuroblastic tumors may differentiate, whereas concomitantly in the same lesion, the glial stroma may dedifferentiate. Anaplastic glia, as in a glioblastoma multiforme, can initiate malignant transformation in alien cells.—Natl Cancer Inst Monogr 46: 51–56, 1977.

Recently, under the auspices of the World Health Organization, a classification was formulated for brain tumors that is being evaluated before it is recommended for international acceptance. It would be inappropriate to discuss this classification prior to this recommendation or to formulate another that might deviate notably from it. Therefore, this paper will consider the problems of classification from the point of view of the Brain Tumor Study Program as it evaluates the efficacy of various therapies for anaplastic gliomas.

The gliomas, which comprise more than 50% of primary intracranial neoplasms, are a heterogeneous group of complex interrelated entities. Traditionally, they have been classified by histologic criteria, with or without recognizing that taxonomic nomenclatures impose rigid terms upon protean systems (*1–8*). Some of the biologic characteristics of the gliomas underscore their protean nature and have immediate relevance to prognosis.

First, I want to emphasize that the glial tumors, whether of astrocytic, oligodendroglial, or ependymal origin, are a spectrum rather than a conglomerate of individual entities. Thus the well-differentiated astrocytoma represents one extreme of the astrocytic series and the markedly anaplastic glioblastoma multiforme represents the other. Between, a succession of lesions is found with gradations of anaplasia and varied morphologies. Histologically, the transition through this spectrum is marked by increased cellularity and cellular pleomorphism, by the appearance of necrosis and pseudopalisading, and by the presence of increased vascularity and endothelial-cell proliferation. The terms applied to this sequence are arbitrary and their application is necessarily subjective. Some investigators prefer to subdivide the spectrum into four categories (*4, 5*). Simplicity dictates three: two extremes, the well-differentiated astrocytoma and the markedly pleomorphic glioblastoma multiforme, and a middle group which requires a broad term. We prefer anaplastic astrocytoma because it is descriptive of the biologic attributes of this group (*2*). Considerable evidence shows that gliomas can originate at any level of the spectrum and that on occasion, but not predictably, many descend but none ascend this declivity of anaplasia (figs. 1–3).

Second, anaplastic glia by their presence alone may initiate malignant transformation in alien cells. This phenomenon is unique to the glia and has broad implications for the entire field of neoplasia. A glioblastoma that contacts the dura or the adventitia of blood vessels infiltrates these mesenchymal structures and characteristically induces exuberant fibroblastic or endothelial cell proliferations. This hyperplastic response may become sarcomatous. The lesion is then a gliosarcoma (*2*) as depicted in figure 4.

We often observe that oligodendrogliomas are colonized by focal proliferations of astrocytes or ependymal cells and that ependymomas often contain aggregates of oligodendroglia. Since in these instances the secondary constituents are also glia, we cannot determine whether they were acquired by annexation and hyperplasia or through divergent differentiation of the original anaplastic glia. Descriptively, the term mixed glioma is appropriate.

The third phenomenon that deserves consideration, the propensity of neuroblastic tumors to differentiate spontaneously into mature ganglionic lesions, perhaps is of even greater significance and, unfortunately, its modus operandi is equally obscure. Remarkably, this biologic event

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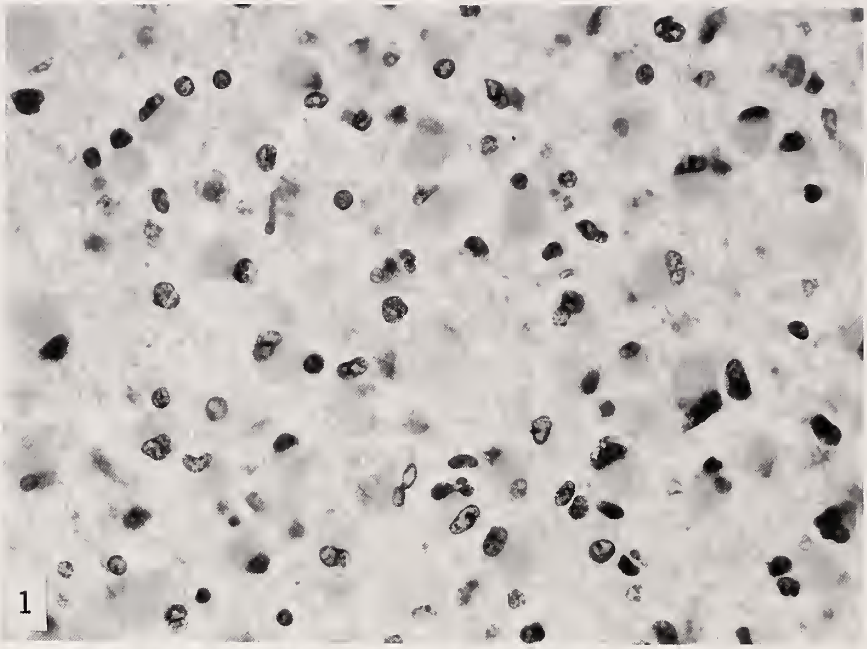


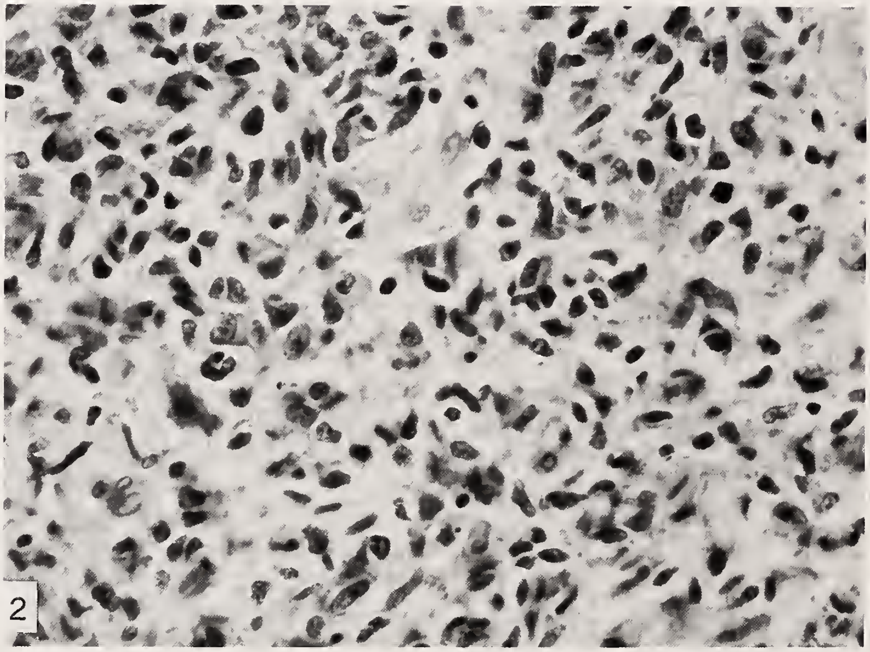
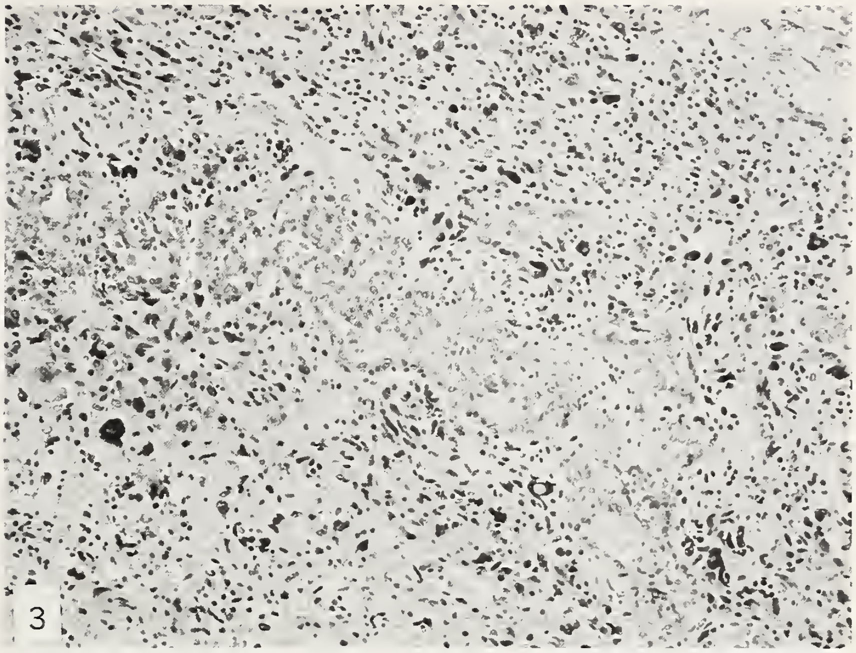


Figure 1.—Astrocytoma is characterized by stellate glia in low density with minimal pleomorphism and an absence of necrosis and endothelial cell proliferation. Hematoxylin and eosin (H & E). × 400

Figure 2.—Anaplastic astrocytoma is characterized by increased cellular density and pleomorphism, but lacks necrosis and notable endothelial cell proliferation. H & E. × 400



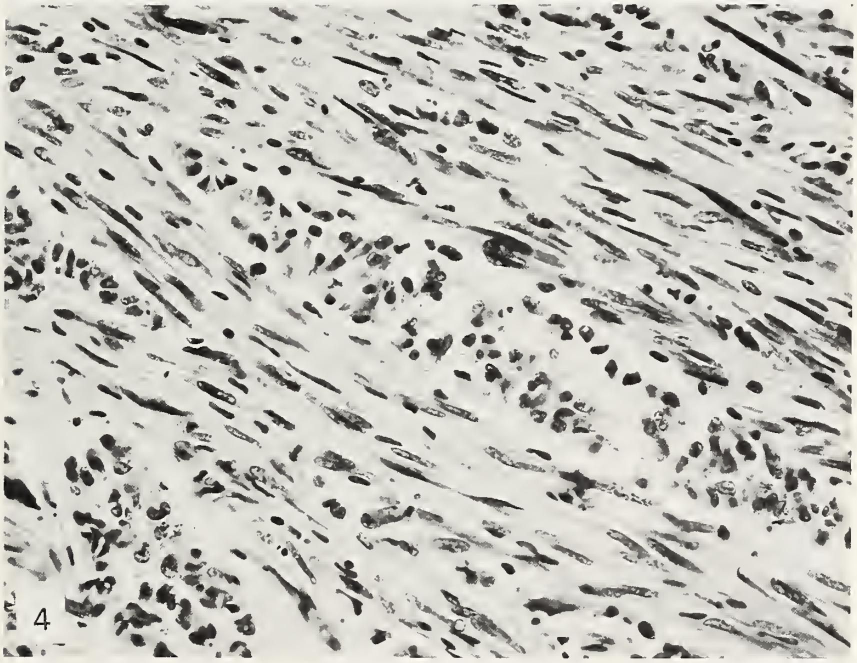
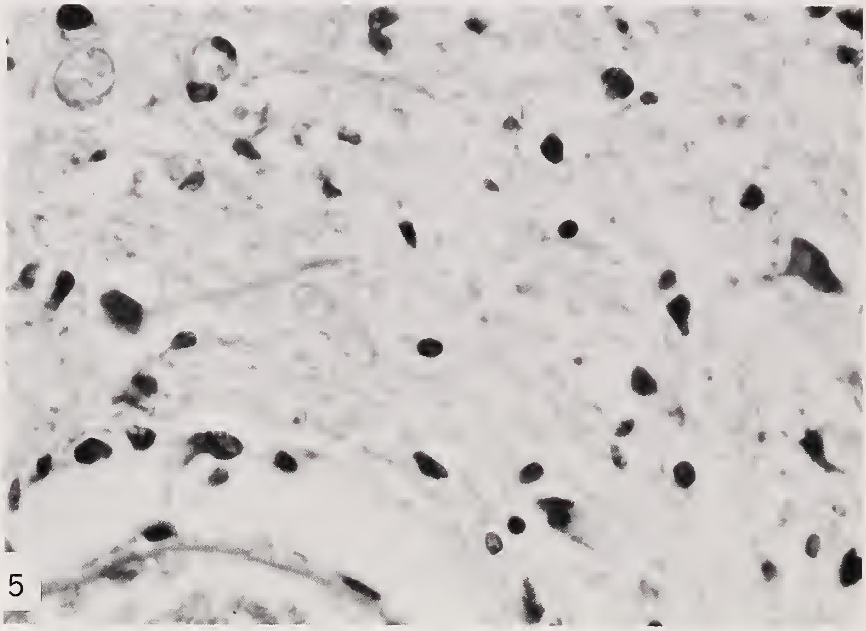


Figure 3.—Glioblastoma multiforme shows high cellularity and pleomorphism, marked endothelial cell proliferation, and serpentine areas of necrosis haloed by mantles of increased cellular density. H & E. × 110 (also Fig. 7)

Figure 4.—A lesion deep in the right frontal lobe of a 68-year-old woman showed (on biopsy) the typical features of a glioblastoma multiforme. Four months later at postmortem examination, the glioma abutted against the dura. Discrete 0.5–2 cm nodules of homogenous fibrosarcoma were attached to the dura, and the mantle of tissue that had replaced the underlying cortex was composed of intermingled anaplastic glia and fibroblasts. The term "gliosarcoma" was applied to this lesion. H & E. × 250



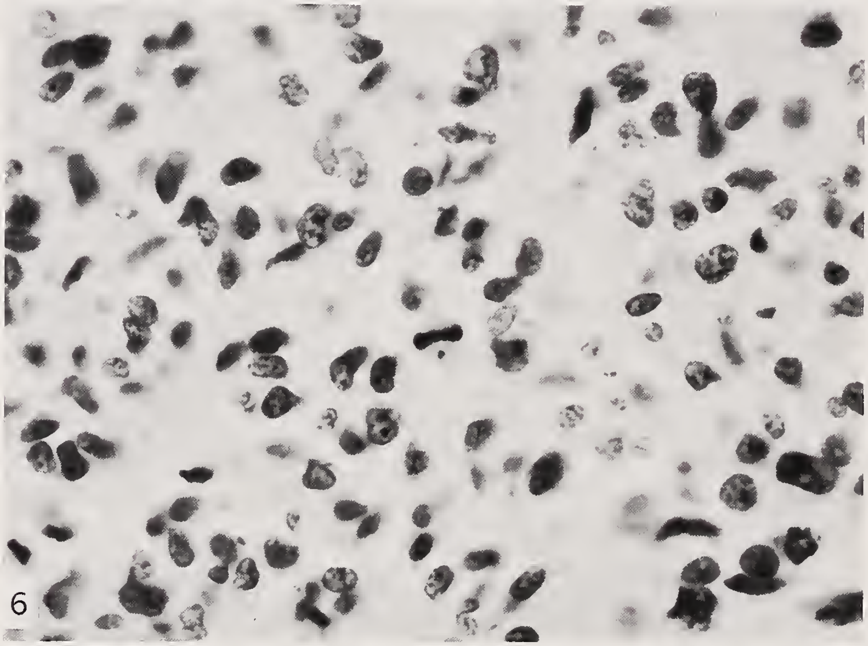
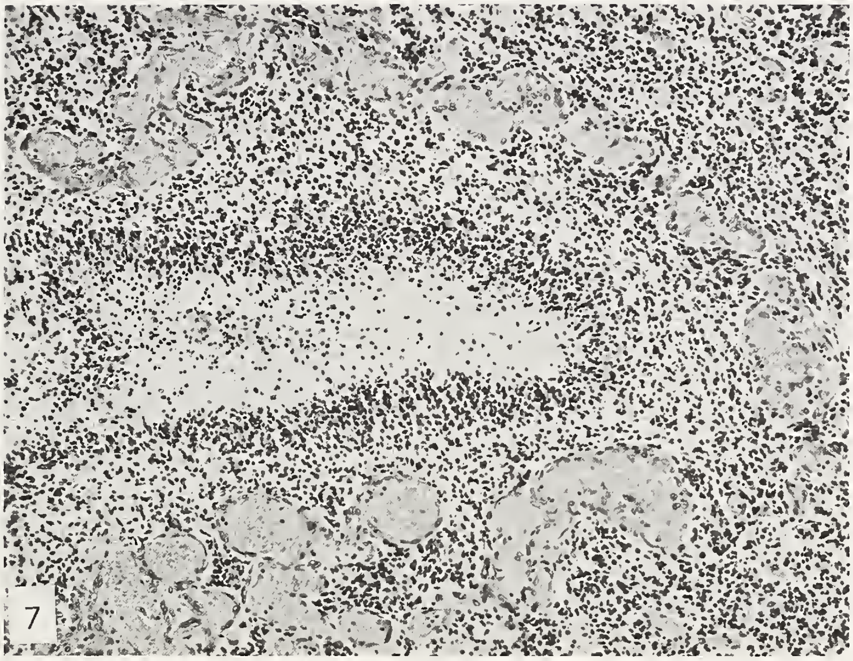


Figure 5.—Divergent phenomena of differentiation of neuroblastoma and dedifferentiation of glia occurred simultaneously in a lesion within the medulla of a 10-year-old boy. When biopsied as a protrusion into the cerebellar–pontine angle, the lesion was composed of undifferentiated neuroblasts and a stroma of well-differentiated glia. H & E. × 100

Figure 6.—The patient discussed in figure 5 died 3 months after the biopsy as a result of extensive leptomeningeal spread at the base of the brain and about the spinal cord, as well as ependymal implantation within the lateral and third ventricles. These metastatic lesions were glioblastomata multiforme, densely cellular, and markedly anaplastic. H & E. × 760



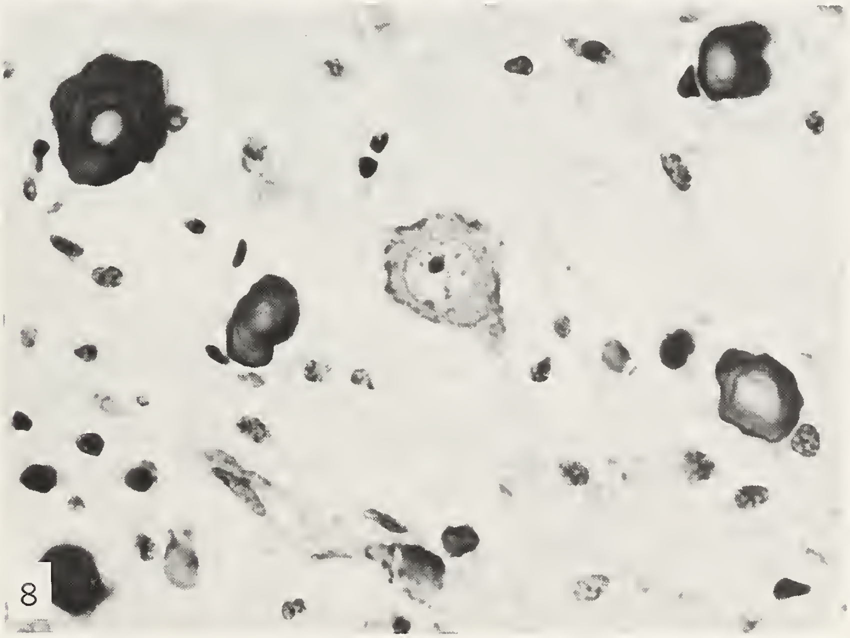


Figure 7.—Extensive necrosis with pallisading and prominent endothelial cell proliferation characterized these implants. H & E. × 100

Figure 8.—Parent lesion in the medulla, a ganglioglioma, was now formed of well-differentiated neurons with much calcification. H & E. × 575 can take place in close temporal and spatial relationships with its converse, the dedifferentiation of astrocytic cells. Thus a ganglioneuroblastoma that contains an admixture of poorly differentiated neuroblasts and a stroma of well-differentiated astrocytes might spawn a lethal glioblastoma multiforme that infiltrates outward from the then well-differentiated parent ganglionic lesion (figs. 5–8).

I do not suggest in this brief discussion another classification of brain tumors but wish to emphasize that the application of any classification must take cognizance of the protean nature of these neoplasms. Being anaplastic, the cells of gliomas have abandoned the unifying and highly sophisticated principles that govern normal cells of the nervous system. The result is loosely constrained heterogeneity. Therefore, in classification, we must be mindful that the assignment of a specific name to a lesion does not in itself individualize that neoplastic process.

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Pathology of Radiation Damage to the Normal Brain of the Monkey[1]

William F. Caveness[2]

ABSTRACT—Model I: A portion of the monkey brain, the right occipital lobe, was exposed to 3,500 rads of orthovoltage radiation in a single dose. This demonstrated a) the delayed, 4 to 5 months, massive break in blood–brain barrier with extravasation of plasmatic fluid throughout the right hemisphere causing gross brain distortion, and *b*) after a protracted course, a remaining impairment in function extending beyond the irradiation lesion. Model II: The whole brain was exposed to supervoltage radiation in single doses of 1,000, 1,500, and 2,000 rads, respectively. This demonstrated *a*) the lack of effect from 1,000 rads; *b*) the wide scatter of necrotic lesions in the forebrain white matter from 1,500 rads at 6 months, followed by confluent necrosis in the cerebral hemispheres at 12 months; and c) the numerous focal lesions throughout the brain from 2,000 rads, with the lesions in the brain stem precluding survival beyond 6 months. Model III: The whole brain was exposed to fractionated doses of 4,000, 6,000, and 8,000 rads in 4, 6, and 8 weeks, respectively. This demonstrated a) the lack of effect from 4,000 rads; *b*) the wide scatter of small focal lesions at different stages of breakdown and repair accompanied by focal edema from 6,000 rads at 6 months, followed by more of the reparative processes including mineralization of focal lesions, and widespread telangiectasia, at 12 months; and c) with 8,000 rads, at 6 months, focal lesions similar to those from 6,000 rads, but at 12 months, gross brain loss from coalescing necrosis.—Natl Cancer Inst Monogr 46: 57–76, 1977.

To set forth observations from our laboratory that may be helpful in anticipating and perhaps understanding the delayed effects from ionizing irradiation in the normal brain of man, we selected the following findings from three modes of brain irradiation in the young adult *Macaca mulatta.*

MODEL I

In the first model, the right occipital lobe of 24 animals was exposed to 3,500 rads of orthovoltage radiation in a single dose. The source was a General Electric Maxitron X-ray machine, operating at 250 kilovolt peaks and 30 milliamperes. The target to skin distance was 23 cm and the dose rate was 300–350 rads/minute. A 0.5-mm copper filter and an aluminum parabolic filter resulted in an X-ray beam having a half-value layer equal to 2.15 mm copper.

The early ultrastructural changes were subtle, implicating glycogen metabolism, and perhaps enzyme systems, as reflected by altered lysosomes.

With routine histology and Golgi-Cox preparations, the sequential changes before 20 weeks were limited to minimal, scattered astrocytic or microglial reactions, with occasional perivascular collections of mononuclear cells. At approximately 12 weeks, there were detectable though not pronounced alterations in dendritic arbors and, to a lesser extent, a loss in cell bodies. From 20 to 24 weeks, we observed an abrupt breakdown in neural tissue, represented most clearly in focal areas of myelin destruction, accompanied by proliferative and degenerative changes in astrocytes, microglia, and oligodendroglia, and collections of mononuclear cells. Vascular changes included proliferation, occlusion, degeneration, and hemorrhagic exudates. Around the periphery were hyperplasia of glia, telangiectasia, and an inflammatory reaction. In the following weeks, the focal areas coalesced into wider areas of tissue destruction that were accompanied by reparative processes of varying but incomplete degree (*1*).

As a part of this complex lesion, there was a massive break in the blood–brain barrier, the clinical implications of which could be profound. To delineate this, we gave an additional set of monkeys 2 ml Evan's Blue dye/kg iv 2 hours before they were killed. In these preparations, the irradiated right occipital lobe was swollen and deeply stained. The swelling extended from the irradiated region throughout the right hemisphere, causing gross brain distortion. Histologic sections from the radiation lesion showed large confluent areas of necrosis and smaller areas of focal coalescing necrosis. The blood vessels had varying degrees of endothelial proliferation, degeneration of vascular walls, hemorrhagic exudates, and telangiectasia. These changes were accompanied by a moderate to marked accumulation of mononuclear cells (fig. 1). As a prominent

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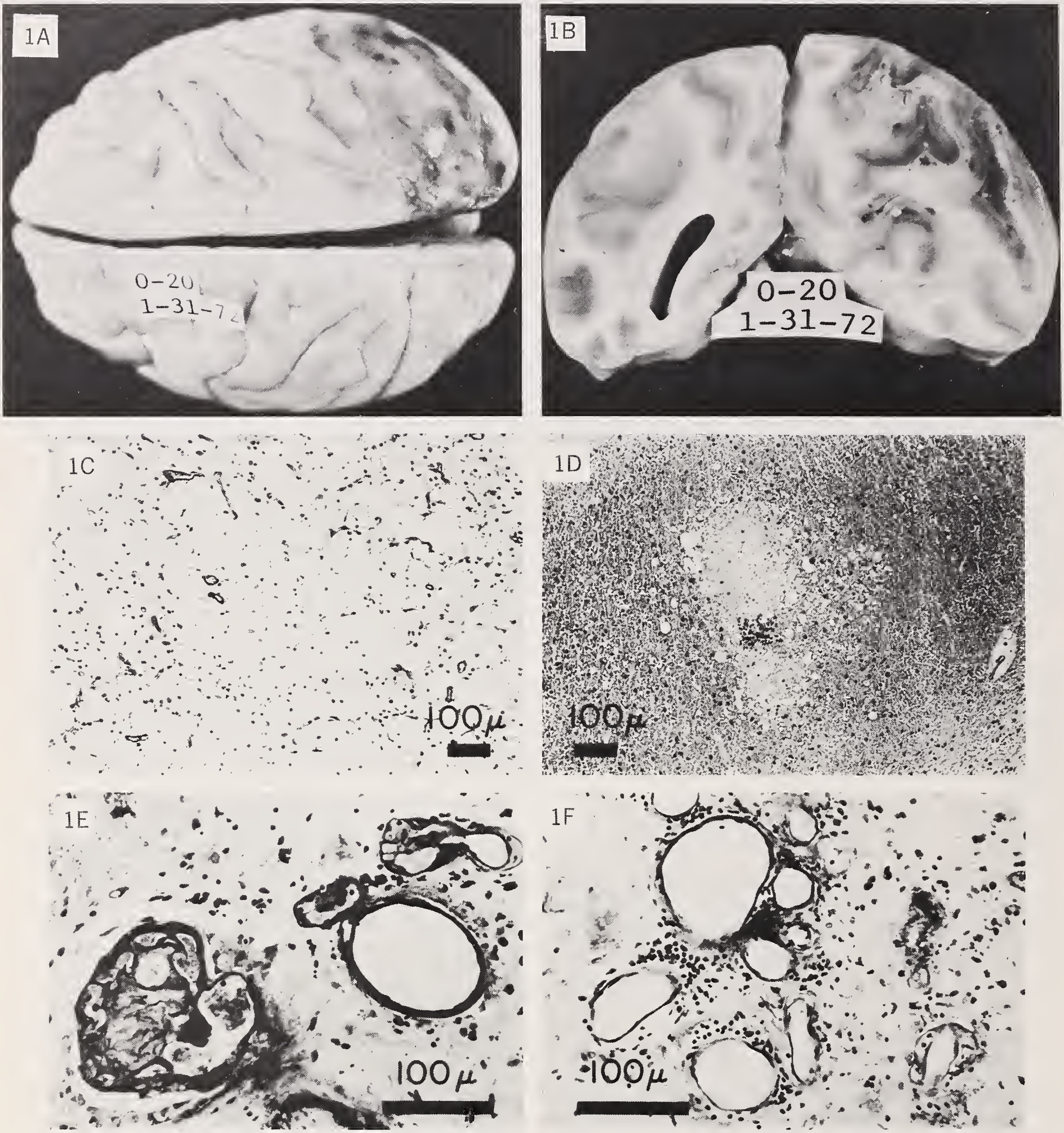


FIGURE 1.—A, B) Monkey O-20, killed 20 wk after exposure to 3,500 rads. Irradiated right occipital lobe is swollen and deeply stained with Evan's Blue. The swelling extends throughout the right hemisphere, causing gross brain distortion. C, D) From sections through the irradiated occipital lobe showing examples on the left of confluent necrosis and on the right patches of tissue loss that have begun to coalesce. H & E. E) Fibrohyaline proliferation in small vessel walls with recanalization. F) Cluster of dilated telangiectatic vessels with surrounding mononuclear cells. PAS.

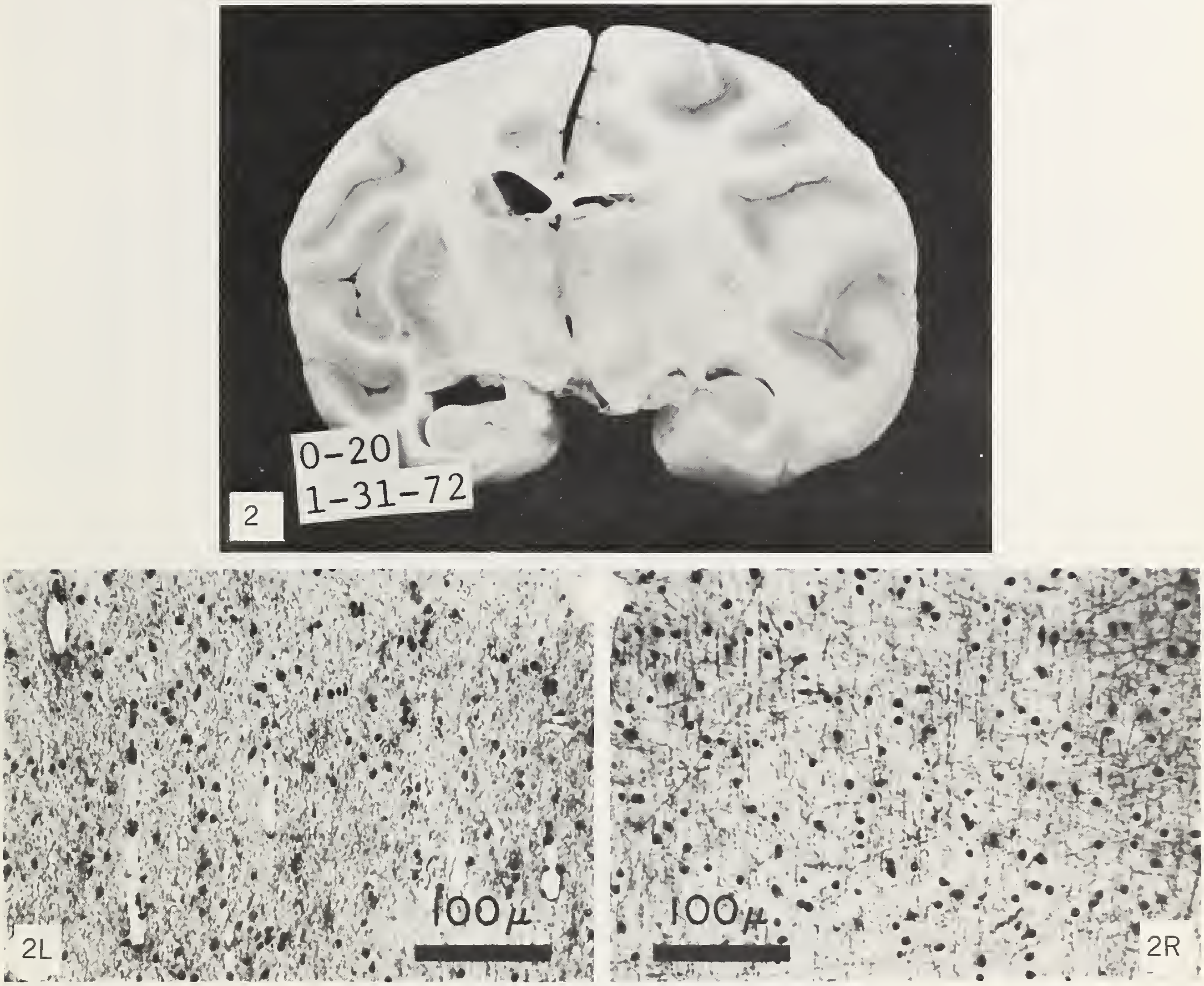


FIGURE 2.—*Top:* Coronal section of the cerebrum at the level of the midthalamus showing edema of the right hemisphere, without staining, dilation of left lateral ventricle, and compression of the left hemisphere. *Bottom:* Photomicrographs from coronal sections through the cerebrum of monkey O-20 at the level of the lateral geniculate bodies, anterior to and well beyond the radiation lesions. *L* and *R* are from homologous areas of white matter within the middle temporal gyrus of the left and right hemispheres, respectively. On the *right,* the fibers are separated by plasmatic fluid that has migrated from the radiation lesion. PAS.

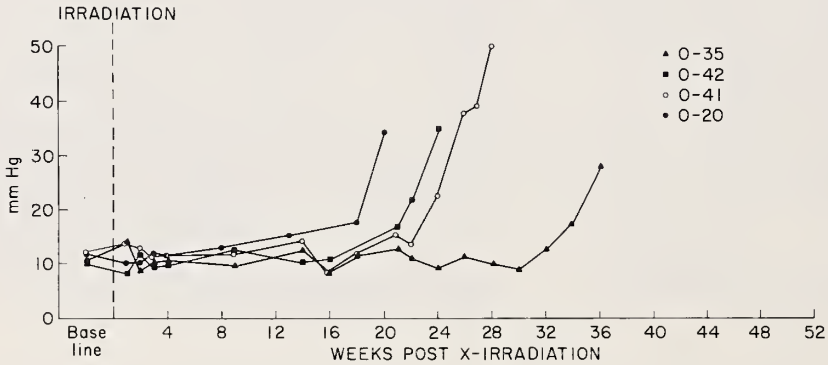
secondary effect, plasmatic fluid that had migrated from the break in the blood–brain barrier had separated fibers in the white matter well beyond the radiation lesion (fig. 2).

To monitor the changes in cerebral spinal fluid pressure that were due to the break in the blood–brain barrier and consequent brain swelling, we used an indwelling catheter–reservoir system that permitted sequential measurements (*2*). The catheter (0.065 cm outer diameter, 0.030 cm inner diameter), made of medical-grade silicone rubber, was inserted in the spinal subarachnoid space at L 2–3 and advanced to the region of the cisterna magna. The caudal end was connected to a side-inlet reservoir (1.5 cm in diameter) that was then secured beneath the skin. For the cerebrospinal fluid (CSF) measurements, the animal was placed in a prone position with the head fixed, the limbs comfortably restrained, and the eyes covered. With sterile technique, a 22-gauge needle connected to a closed manometric system was passed through the skin into the reservoir. Pressure measurements above the level of the heart were made over a 30-minute period in a quiet environment to minimize the effects of agitation.

Six weeks after the implantation of the catheter–reservoir system, the right visual cortex and the immediate subjacent white matter in 12 monkeys were exposed to 3,500 rads of X-irradiation. Two unirradiated similar preparations served as controls. In 8 of the 12, the pressure curves indicated a delayed abrupt rise that might take place as early as 18 weeks or as late as 36 weeks following the exposure (text-fig. 1; fig. 3). The pressure increase achieved an elevation of three or more times base line within a period of 2 weeks. This abrupt increase was reflected in papilledema (4/8), oculomotor palsies (3/8), behavioral blindness (4/8), gross depression in visual evoked response (8/8), and predominantly delta activity in the background electroencephalographic activity (8/8). The 4 animals that developed behavioral blindness are of special interest. The unilateral destruction of the visual cortex and the bilateral papilledema were insufficient to account for this, since the blindness and papilledema did not always occur in the same animal. We believe the blindness was the result of gross distortion of the visual pathways within the brain, i.e., the swelling in the right hemisphere and the compression in the left, implicating particularly the lateral geniculate bodies (fig. 3).

Three of the 12 animals showed only a mild elevation in CSF pressure between the 20th and 36th week. These were never more than twice base line. Observed for 52 weeks, none of these 3 animals showed abnormal clinical signs, although all showed mild depression of the visual evoked response (VER) on the right side. When killed, all showed some staining with Evan's Blue, but they had less pronounced histologic changes of the irradiated site than those seen in the previous group of 8 monkeys.

A single monkey showed an appreciable but more gradual increase in CSF pressure, three times base line at 22 weeks with a return to, and below, base line from the 40th week. Papilledema from the 18th to 30th week and absent VER from the 25th to 30th week were noted, then a gradual recovery of the latter during the remainder of the 52 weeks of observation occurred. When the animals were killed, no staining with Evan's Blue was seen in the brain, but rather a shrunken fibrotic lesion in the irradiated right occipital lobe (text-fig. 2, fig. 4). The lateral geniculate body on the right showed cell loss



TEXT-FIGURE 1.—CSF pressure curves representing the delayed, abrupt rise 18–36 wk after exposure.

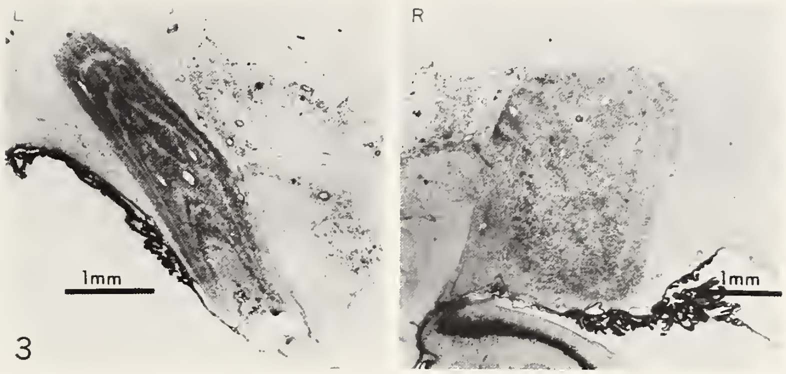


Figure 3.—An example of marked distortion within the visual pathways accompanying an abrupt increase in CSF pressure. Left and right lateral geniculate bodies 22 wk after X-irradiation. Neither shows recognizable cell loss. The *right* shows edema involving all cell layers, with loss in demarcation between lamina; the *left* is compressed and elongated with the cells more compactly arranged, though the lamina can be clearly discerned. Cresyl violet.

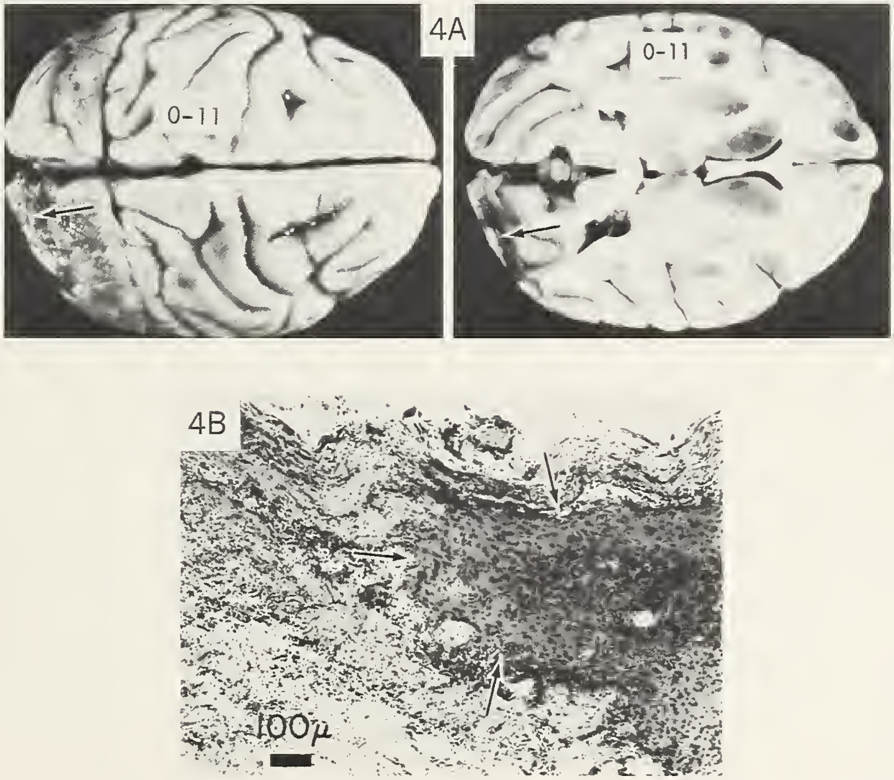
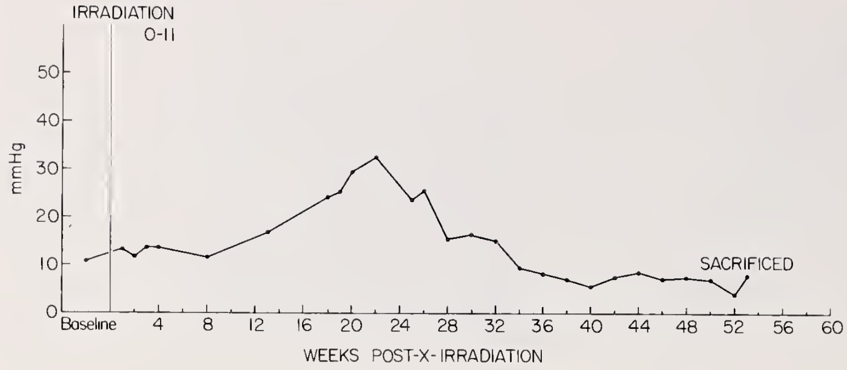


Figure 4.—A) Vertex and horizontal section view of the brain from O-11. Both show a shrunken fibrotic lesion in the irradiated, right occipital lobe, with no Evan's Blue staining. Beyond the healed lesion, no brain swelling or distortion appears. B) Photomicrograph of a section from the gliotic, atrophic lesions in the right visual cortex. A wedge-shaped remnant of a gyrus (*arrows*) is seen encased in a thick meningeocerebral cicatrix. H & E.



TEXT-FIGURE 2.—CSF pressure curve for O-11 exhibiting a steep rise and decline, remaining below base line from the 40th wk. Of the 4 monkeys observed for 52 wk, this was the only one with complete recovery from an elevated CSF pressure.

involving all lamina. Only the most ventromedial and ventrolateral parts were spared. These changes were evidently the remnant of pronounced edema in the right hemisphere, with a probable contribution from retrograde degeneration from the radiation lesion.

As an extension of the observations from this model, we determined the regional cerebral blood flow (rCBF) to gain evidence of metabolic impairment, not only within the developing lesion but also throughout the brain (*3*). For this, 8 monkeys were used: 1 was killed at base line, 3 during the phase of development of increased CSF pressure, 1 at its peak, 1 upon its recovery, and 2 unirradiated animals served as controls. The clinical and laboratory findings at the time of the rCBF determination are indicated in table 1.

The blood flow was determined by the [^{14}C]antipyrine method of Reivich et al. (*4*), which may be summarized as follows. After the last recording of cisternal pressure, CSF examination, funduscopic photography, EEG, and VER, the abdominal aorta and the inferior vena cava were catheterized, respectively, via the femoral artery and vein under general anesthesia with halothane and air. After full recovery from the anesthesia, approximately 200 μCi [^{14}C]antipyrine/kg body weight, a diffusible radioactive tracer, was continuously infused for 60 seconds via the femoral vein. During the infusion, about 20 drops of

TABLE 1.—*Clinical and laboratory findings at time of rCBF*

Monkey	Irradiation to rCBF, wk	Clinical	Optic fundi	Electroencephalogram (EEG)		CSF protein, mg/100 ml	CSF lactic dehydrogenase, U/ml	Brain swelling	Tentorial herniation	Foramen magnum herniation	Dilatation of contralateral ventricle
				Slow waves	VER						
Z-9	19	Normal	Normal	None	Normal	Normal	Normal	None	None	None	None
Z-10	25	Loss of appetite	Normal	Focal	Unilateral suppression	Normal	84	+	None	None	None
Z-14	27	Loss of appetite	Mild papilledema	Diffuse	Unilateral suppression	Normal	176	++	+	+	+
Z-22	29	Vomiting, lethargy, clumsiness, blindness	Mild papilledema	Diffuse	Bilateral suppression	142	130	+++	+	+	+
Z-24	28	Vomiting, lethargy, clumsiness, blindness	Mild papilledema	Diffuse	Bilateral suppression	No measurement		+++	+	+	+
Z-16	40	Loss of appetite, clumsiness[a]	Mild papilledema[a]	Focal	Bilateral suppression	135	107	++	None	None	+

[a] Symptom was noted at 29 weeks, the peak of CSF pressure.

arterial blood were collected from the femoral artery for the determination of the arterial concentration curve of [^{14}C]antipyrine. One minute after the start of the infusion, the monkey was decapitated and the whole head immersed in a Freon bath, chilled by liquid nitrogen to −100° C. The head was then sectioned in the horizontal plane at 30-μ thickness with a Jung K-type microtome at a cryostatically controlled temperature of −20° C. Approximately 120 serial sections from each brain were collected on plastic tape and dried on a hotplate at 60° C. The brain slices were then placed on single-coated, blue-sensitive medical X-ray film with standard disk plates that had been calibrated for brain slices of 30-μ thickness. The exposure time was 5–7 days and the films were developed by hand. The concentration of [^{14}C]antipyrine in the brain was determined from the optical density of the macroautoradiograms and of the standard disk plates. From the determination of the concentration of the [^{14}C]antipyrine in the arterial blood and the concentration of the tracer in the macroautoradiographs, the value of the actual regional cerebral blood flow in milliliters per gram per minute was calculated from the formula devised by Kety (*5*).

The major changes in rCBF are schematically represented, and the total results summarized in text-figures 3–5. From these data, it is clear that as the CSF pressure increased the blood flow decreased. This decrease was at first greater in the right hemisphere, the side of the lesion, and in the white matter more than the gray matter. However, at the highest pressures, the blood flow was decreased bilaterally in both white and gray matter. Of perhaps grave significance was the only partial recovery in blood flow in the monkey whose intracranial pressure had returned to normal. Whereas there had been greater improvement in gray than white matter, a diffuse reduction in blood flow remained, which suggested a long-term impairment in cellular metabolism and/or blood-flow regulatory mechanisms.

What principles are demonstrated by this first model, i.e., the irradiation of a portion of the brain with a single dose of 3,500 rads?

1) There is a protracted delay of weeks or months before there is overt breakdown of neuronal tissue.

2) Once this starts, the destructive process proceeds quickly.

3) A prominent feature is a break in the blood–brain barrier that not only contributes to the local destruction but through the migrating plasmatic fluid can damage remote areas, either directly through separation and compression of neuronal elements, or indirectly through distortion of brain structures and impairment of CSF circulation.

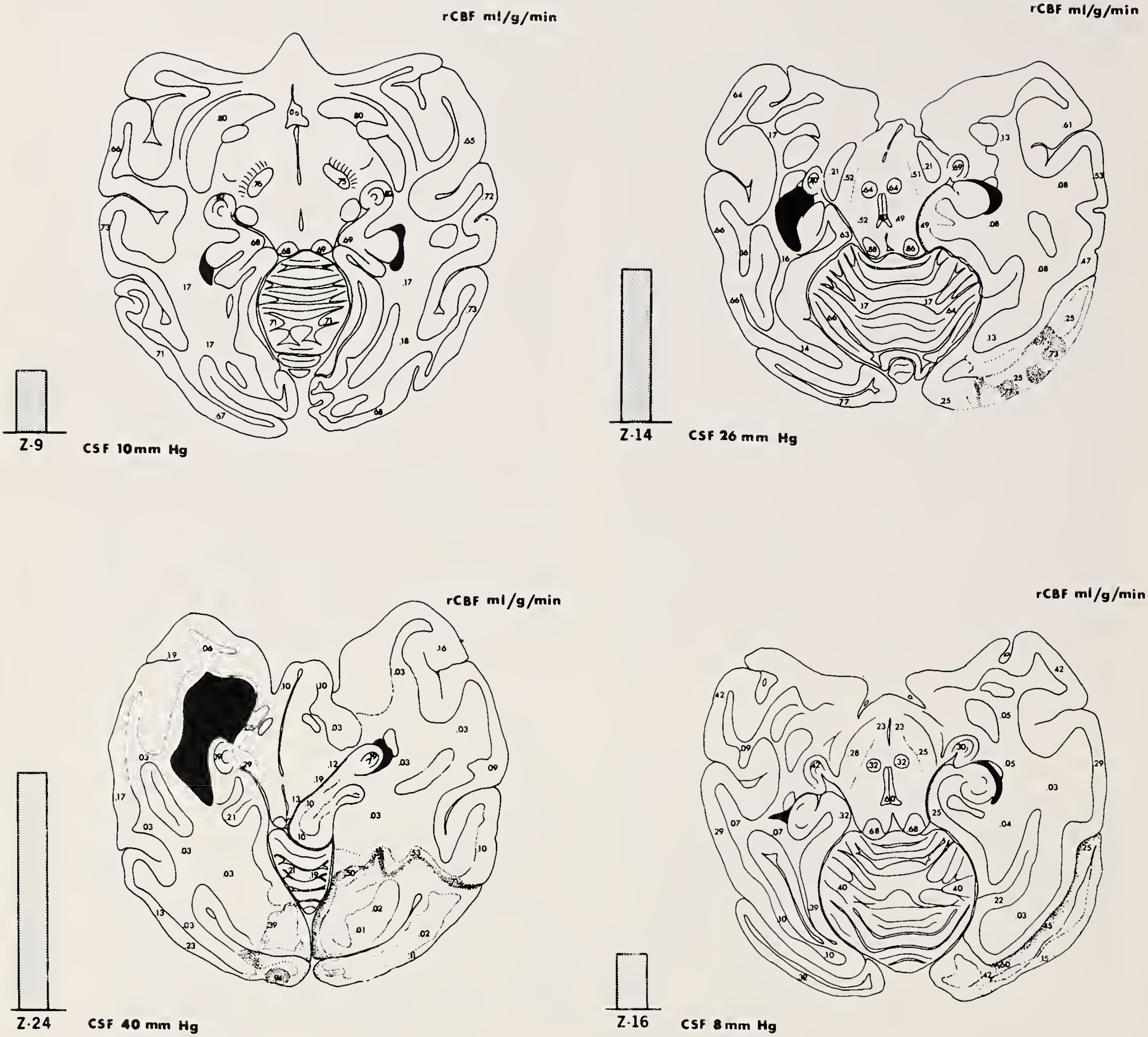
4) The lesions seldom, if ever, heal in the time span studied. In those instances in which the reparative processes "seal off" the lesions, focal, and perhaps diffuse, impairment in neuronal function may remain as a remnant of the destructive processes.

MODEL II

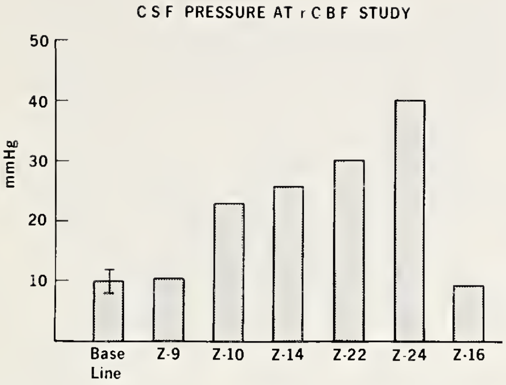
In the second model, the whole brain was subjected to a single exposure of supervoltage irradiation. Three groups of 4 monkeys each received 1,000, 1,500 and 2,000 rads, respectively. The source was a linear accelerator with an electron beam energy of 20 MeV that after collimation was converted to photons by tantalum foils and flattened by a lead disk to provide a nearly constant radiation field. The body was shielded with 3–8 inches of lead, except for an opening limited to the external borders of the brain, as determined by routine skull X-ray films. Dosimetry by thermoluminescent dosimeters and ionization chambers indicated a variation of less than 5% throughout phantom brains, unilaterally irradiated from the left side. The dose rate at midplane was 200 rads/minute. The source axis distance was 150 cm. The irradiations were done at the Armed Forces Radiobiology Research Institute. The monkeys were killed at 26, 52, 78, or 104 weeks after irradiation; the brains were fixed by perfusion with paraformaldehyde, embedded whole in celloidin, and cut in serial sections at a thickness of 35 μ. Every 20th section was stained for myelin by the Loyez method and the adjacent section for nerve cells and glial nuclei with the Nissl method. An additional set, 100 sections apart, was stained with hematoxylin and eosin (H & E) and periodic acid–Schiff (PAS), for vascular and other structures. The number of focal and confluent lesions are indicated in table 2.

Those with the whole brain exposed to 1,000 rads in a single dose demonstrated no clinical signs, other than inconstant depression in the VER that was observed in monkey L-26.

In contrast, those irradiated with 1,500 rads showed progressive neurologic abnormalities. Monkey L-32, killed at 26 weeks, had diminished motor activity from the 24th week. L-31, killed at 52 weeks, showed myoclonic jerks of the trunk and proximal joints of limbs, easily provoked by rattling the cage, that began at 26 weeks. The animal also experienced depression in the VER from the 26th week and high amplitude slow



TEXT-FIGURE 3.—Schematic representation of horizontal sections from monkey undergoing various degrees of brain swelling. The *numbers* within the diagrams indicate the milliliters of rCBF per gram per minute, determined by the [^{14}C]antipyrine method. A) In Z-9, with no elevation in CSF pressure, the rCBF's for gray and white matter were within the limits of the controls. B) Z-14, with a CSF pressure better than twice base line, had a distinct reduction of blood flow in the swollen right hemisphere extending well beyond the radiation lesion, predominantly in the white matter. C) In Z-24, with a CSF pressure some four times base line, the reduction in blood flow was pronounced throughout the right and the left hemisphere in both white and gray matter. Along the margin of the radiation lesion there was an expected increase in flow. D) Z-16 had experienced a CSF pressure of 24 mm Hg at 29 wk but had a pressure of 8 mm Hg when killed at 40 wk. Even with this recovery, a remnant of the edema and a diffuse reduction in rCBF remained.

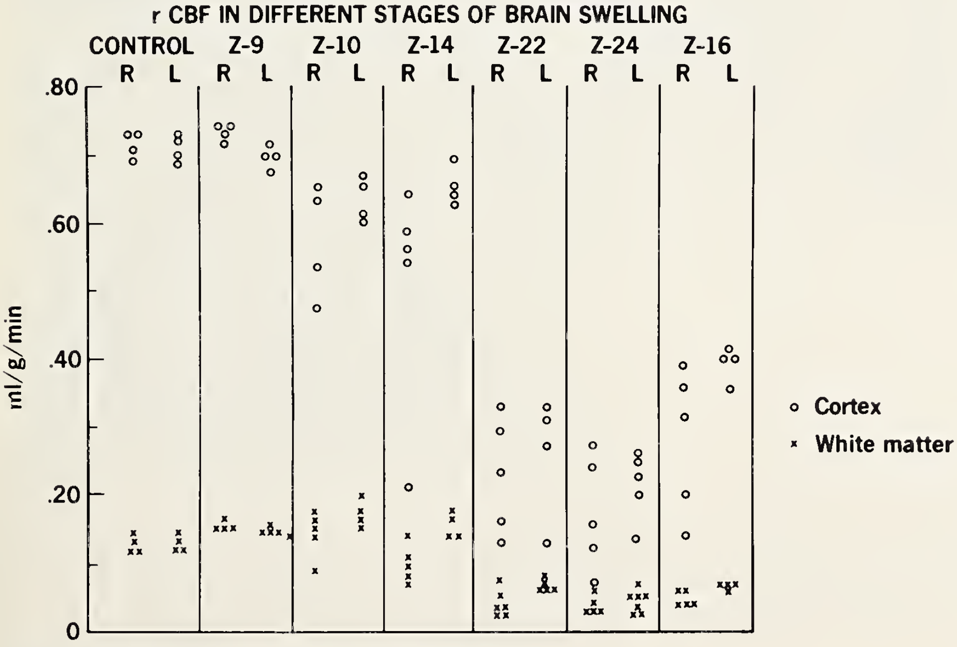


Text-figure 4.—Bar graph of CSF pressure at time of rCBF determinations.

waves (delta activity) in the background EEG from the 30th week. Ataxia was noted in the lower extremities at 30 weeks, and at 50 weeks, loss of visual acuity was suspected because of optic disk pallor and a poor response to objects placed directly in his visual field. L-30, killed at 78 weeks, showed early papilledema from the 10th through the 14th week that receded by the 18th week. The VER was depressed from the 18th week, with minimal response from the 38th to the 50th week. High amplitude slow waves were prominent in the EEG from the 30th to 52d week. From the 28th week, voluntary motor activity was limited; the animal squatted in the cage, exerting himself only for feeding. L-33, killed at 102 weeks, experienced a gradual reduction in VER until stabilization at low amplitude at 56 weeks. Impairment in VER latency occurred from the 94th week. Optic disk margins were blurred at the 23d week, but at 100 weeks papilledema was pronounced. Dilated and nonreactive pupils with behavioral blindness were noted from the 100th week. Motor activity was diminished during the latter part of his course and from the 101st week he could not stand. From this point, he had difficulty eating and occasionally retched.

With whole brain exposure to 2,000 rads, none of the monkeys survived beyond 26 weeks. Their deteriorating condition included crippling loss in motor power and dexterity, with finally an inability to ingest food. Three of the 4, L-35, L-37, and L-34, had pronounced papilledema beginning in the 8th week; the fourth, L-36, had blurred disk margins in the 12th week but this was less evident at the time they were killed. VER was impaired in all monkeys, beginning between the 8th and 16th weeks; delta activity was pronounced in 1, L-36.

The brains of those monkeys exposed to 1,000 rads showed no gross pathologic abnormality. On



Text-figure 5.—Summary of rCBF determinations for cerebral cortex and white matter. Each symbol represents the average of the measurements for the frontal, parietal, temporal, and occipital regions, respectively.

TABLE 2.—*Distribution of focal lesions after single exposure*[a]

Structure	1,000 rads				1,500 rads				2,000 rads			
	26 wk	52 wk	78 wk	104 wk	26 wk	52 wk	78 wk	102 wk	19 wk	20 wk	20 wk	26 wk
	L-28	L-26	L-29	L-27	L-32	L-31	L-30	L-33	L-36	L-35	L-37	L-34
Cerebral cortex						Confluent	5	1				
Corona radiata					31	Confluent	Confluent	Confluent	303	627	122	669
Centrum semiovale					32	Confluent	Confluent	Confluent	213	406	125	446
Corpus callosum						Confluent	2	Confluent	6	67	12	55
Fornix						4				20	10	7
Internal capsule						Confluent	18	25	65	53	21	66
Thalamus						Confluent	8	39		4	22	22
Hypothalamus						2		1	12	3	12	37
Subthalamus						Confluent		6	6	2	1	7
Optic tract									4	2	8	16
Basal ganglia						Confluent	3	16	12	1	3	15
Amygdala									1		6	
Archicortex						Confluent			4	14	16	46
Tectum						12		13	2			4
Tegmentum, mesencephalon						57	10	53	53	27	49	68
Tegmentum, pons						23	4	31	46	39	38	120
Basis pontis						13		4	95	95	108	150
Medulla						2	1		49	22	12	25
Cerebellar white matter						52	12	7	27	79	49	73
Cerebellar roof nuclei						3	1		29		1	1
Total	0	0	0	0	63	(168)	(74)	(196)	927	1,461	615	1,827

[a] *Numbers in parentheses* are less significant because of the confluence that wipes out individual lesions.

serial sections, no lesions were seen, although occasional scattered minute mineral deposits were noted.

The brains of the monkeys subjected to 1,500 rads that were killed at or beyond 52 weeks revealed evidence of swelling and symmetrically dilated ventricular systems. When sectioned, monkey L-32, killed at 26 weeks, showed scattered focal lesions in the forebrain white matter. These consisted of discrete, minute areas of necrosis, in some of which mineral deposits were found. When L-31 was killed at 52 weeks, we saw widespread areas of coalescing necrosis involving primarily the white matter of the cerebral hemispheres, along with focal lesions in the brain stem and cerebellum (fig. 5). Although there was some mineralization in the focal lesions, little was found in the confluent areas of necrosis. Vascular changes included amorphous, subendothelial deposits in the small arteries and capillary telangiectasia. L-30, killed at 78 weeks, had a pattern of confluent and focal necrosis similar in kind to but less in degree than that in L-31. On gross examination, atrophic as well as edematous changes and cerebellar tonsillar herniation were seen in L-33 when the animal was necropsied at 102 weeks. Histologic examination showed prominent confluent lesions in the forebrain white matter and focal lesions in the central gray and brain stem.

With whole brain exposure to 2,000 rads and no survival beyond 26 weeks, the most striking gross abnormality in the brains from monkeys L-34, L-35, L-36, and L-37, was the symmetrical enlargement of the ventricular system. The leptomeninges were unremarkable and the exit foramina were patent. Upon sectioning, all had a wide scatter of focal necrotic lesions that, though numerous and extensive, had not yet shown the coalescing or confluence that was exhibited after a longer interval (52 weeks) by monkey L-31 with the smaller exposure (1,500 rads). The large number of fresh lesions in the brain stem undoubtedly contributed to inability to survive (table 2, fig. 5). There were also lesions in the cranial nerves that may have interfered with masticatory movements.

The principles to be derived from this second

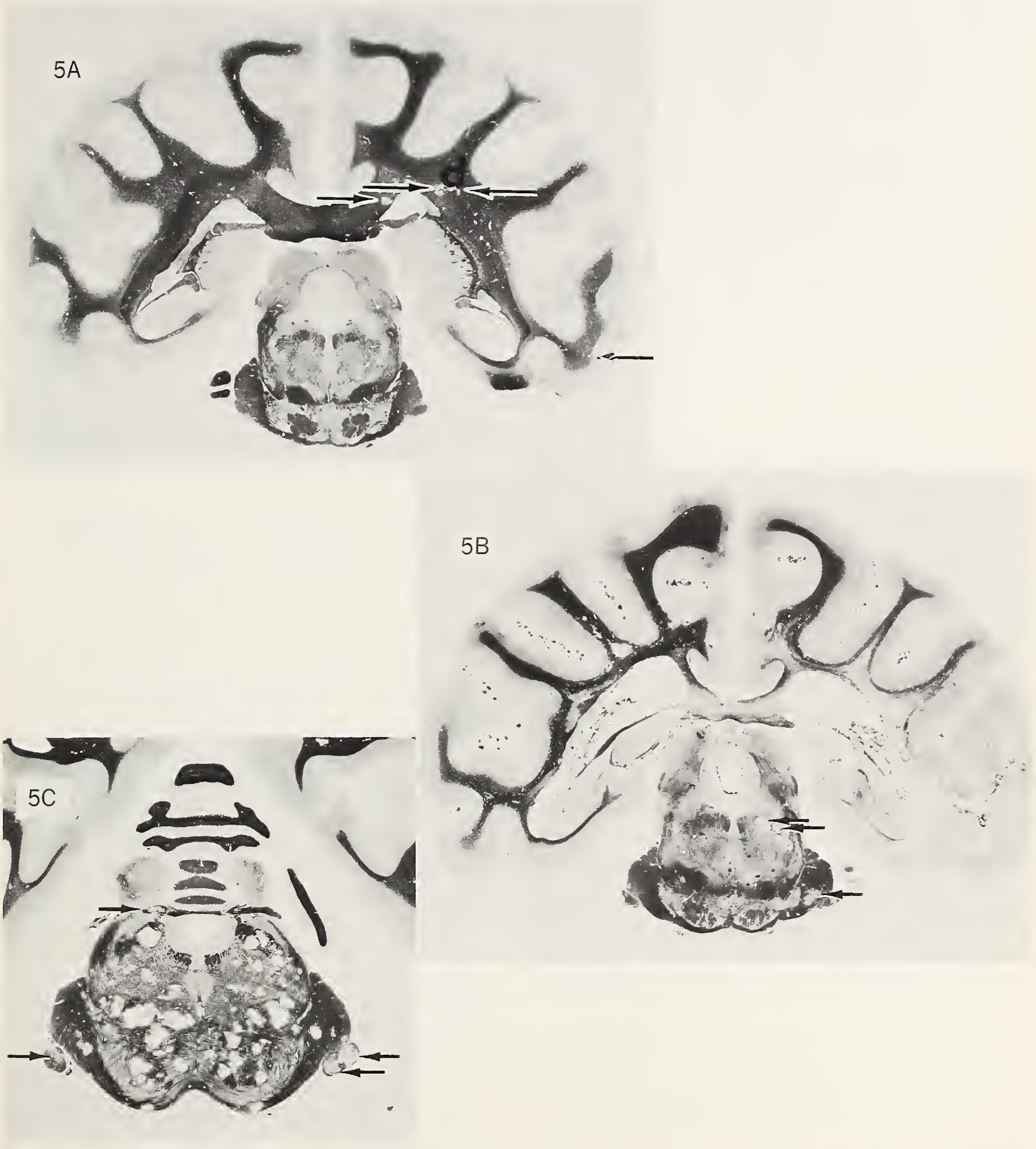


FIGURE 5.—A) Coronal section of monkey L-32, killed at 26 wk following whole brain exposure to 1,500 rads in a single dose; *note* focal areas of necrosis in the forebrain white matter. B) L-31, killed at 52 wk following a similar exposure, shows confluent necrosis in the cerebral hemispheres with some focal lesions in the brain stem. C) L-34, killed at 26 wk following exposure to 2,000 rads; the excessive focal lesions in the brain stem and cranial nerves (*arrows*) can be seen. Myelin. × 16

model are straightforward:

1) Whole brain exposure to 1,000 rads in a single dose causes no lesions in the first 104 weeks following irradiation.

2) Exposure to 1,500 rads causes small focal areas of necrosis in the forebrain white matter at 26 weeks, but a much more extensive involvement at and beyond 52 weeks that includes confluent areas of necrosis in gray and white matter, with focal lesions extending into the brain stem. At and beyond 52 weeks, ventricular dilation, evidently from brain loss is seen.

3) Exposure to 2,000 rads causes such a wide scatter of focal areas of necrosis throughout the brain, including a large number in the brain stem, that survival beyond 20–26 weeks is not possible. All showed atrophic gyri and enlarged ventricular systems.

MODEL III

In the third model, the whole brain was subjected to fractionated exposures of supervoltage irradiation at approximately 200 rads a day, 5 days a week, with the same equipment described for the previous series. To imitate the human exposure of 6,000 rads, now used for malignant gliomas in several medical centers in the United States, we treated 3 groups of 4 monkeys each with 4,000, 6,000, and 8,000 rads in courses of 4, 6, and 8 weeks, respectively (*6*).

The functional changes may be summarized as follows:

Animals that received 4,000 rads had none.

Of those that received 6,000 rads, the VER of monkey L-13, killed at 26 weeks, was altered from the 10th week. L-19, killed at 52 weeks, had pronounced papilledema from the 8th to the 24th week, with optic disk pallor thereafter. High amplitude slow waves in the background EEG were evident from the 24th week, as was an impairment in VER. L-14 died of an intercurrent meningitis at 66 weeks after demonstrating no neurologic signs other than blurring of disk margins from the 18th to the 28th week and loss of appetite at the 65th week. On the other hand, L-16, killed at 78 weeks, showed well-defined papilledema from the 8th to the 16th week, depression in VER from the 16th week, and high amplitude slow waves in the background EEG at 32 and 36 weeks.

Of those that received 8,000 rads, monkey L-20, killed 26 weeks after irradiation, had impairment in the VER from the 8th week and in appetite and motor coordination from the 22d week. L-22 was killed off schedule at 32 weeks because of a rapidly deteriorating clinical condition. Papilledema was pronounced from the 6th to the 16th week. EEG delta activity was apparent from the 24th week. She became increasingly ataxic at 25 weeks; she could hardly walk 5 weeks later, and by 32 weeks she could not stand or sit. L-23, killed at 52 weeks, had impaired VER from the 8th week, with the addition of delta activity from the 32d. Papilledema was noted from the 8th to the 12th week, and optic atrophy was apparent from the 40th week. Pupillary response was decreased at 36 weeks and behavioral blindness was noted from the 48th week. From the 26th week, L-23 had altered motor function and by the time the monkey was killed, an incapacitating loss in strength. The VER of L-21, killed at 78 weeks, was impaired at 20 weeks, markedly depressed at 28 weeks; no response was noted from the 36th week. High amplitude slow waves were apparent in the background EEG from the 32d week. Blurred optic disk margins were noted at 12 and 16 weeks, and optic disk pallor from the 40th week. We observed unequal size in pupils periodically from the 20th week, and no response to light from the 36th week. Impaired visual acuity was suspected from the 32d week and behavioral blindness was observed from the 36th week. At 24 weeks, this animal became ataxic (more apparent in the lower extremities) and by 40 weeks, she would fall when attempting to climb in her cage. In the latter part of her course, diminished motor activity and abnormal posture with the head held in right lateral flexion were seen. Four control monkeys showed no functional or structural abnormalities.

The distribution of the lesions is shown in table 3. Of the animals exposed to 4,000 rads, 1 had a single necrotic lesion in the corona radiata.

Of the animals exposed to 6,000 rads, L-13, killed at 26 weeks, showed 143 lesions scattered throughout the forebrain, predominantly in the white matter of the corona radiata, centrum semiovale, and internal capsule, with a fair number in the central gray matter. These consisted of areas of necrosis 1 mm or less in diameter, attended with varying degrees of vascular, glial,

TABLE 3.—*Distribution of focal lesions after fractionated exposure*[a]

Structure	4,000 rads				6,000 rads				8,000 rads			
	26 wk	52 wk	78 wk	104 wk	26 wk	52 wk	66 wk	78 wk	26 wk	32 wk	52 wk	78 wk
	L-10	L-9	L-12	L-11	L-13	L-19	L-14	L-16	L-20	L-22	L-23	L-21
Cerebral cortex					2			3		3	Confluent	Confluent
Corona radiata	1				93	5	46	176	16	956	Confluent	Confluent
Centrum semiovale					13		13	22	19	593	Confluent	Confluent
Corpus callosum					1				1	24	Confluent	Confluent
Anterior commissure					1							
Fornix									1	35	7	
Internal capsule					14	3	3	2	5	34	Confluent	Confluent
Thalamus					11		1	26		68	Confluent	Confluent
Hypothalamus									2	14	3	
Subthalamus									1	21	6	
Optic tract					2	1				1	1	
Basal ganglia					4	1				20	Confluent	1
Amygdala										1		
Archicortex								1		19		
Tectum					1					18	11	10
Tegmentum, mesencephalon								3	1	84	33	36
Tegmentum, pons						2		2	6	57	21	12
Basis pontis					1			2	11	35	24	13
Medulla										21		2
Cerebellar cortex											2	16
Cerebellar white matter								5	1	80	31	
Cerebellar roof nuclei										5	10	
Total number of focal lesions	1	0	0	0	143	12	63	242	64	2,089	(149)	(90)

[a] *Numbers in parentheses* are less significant; when lesions become confluent, the total number of focal lesions is uncertain.

and macrophage reactions. The individual lesions were of different ages. The most recent consisted of pale areas, with a central core of necrotic brain surrounded by a narrow rim of macrophage response. Slightly older lesions contained numerous macrophages throughout. The oldest had mineral deposits that stained for both calcium and iron (fig. 6). Myelin sections adjacent to the Nissl sections at times revealed corresponding areas of pallor that often exceeded in extent the area of necrosis, suggesting focal "vasogenic" edema. The wide scatter of these lesions is best appreciated with whole brain serial sections, as indicated in the schematic drawings in text-figure 6. These could be missed or their extent underestimated if the brain was examined with routine blocks and thin sections. Adjacent to the punctate lesions are occasional grossly altered vascular endothelial cells. Quite apart from these are areas of capillary proliferation or telangiectasia, 5–8 mm in extent (fig. 7).

The histology at 52 weeks was different from that at 26 weeks. L-19 had only 12 small necrotic lesions, and most of these were mineralized. In addition, there were innumerable minute areas of mineral deposits that may represent remnants of larger necrotic lesions, degenerated vascular walls, or plasma products that now are represented by deposits that stained for calcium and iron. A more active process is reflected in widespread areas of dilated capillaries or telangiectasia, more than were seen at 6 months. L-14, killed at 66 weeks, had 63 small necrotic lesions with varying degrees of associated reactions including mineralization in 49. Also noted were scattered minute mineral deposits bilaterally. L-16, killed at 78 weeks, showed a wide distribution of 242 focal necrotic lesions, with greater extension into the brain stem than was seen in the animals killed at shorter intervals. The accompanying vascular reactions and mineral deposits were, however, more apparent (fig. 8). From the preceding, it appears that monkeys exposed to 6,000 rads have fewer and fewer necrotic lesions with the passage of time, and a progressively larger number are mineralized, which suggests a tendency toward healing. The monkey killed at 78 weeks had more lesions than were found at earlier intervals, but

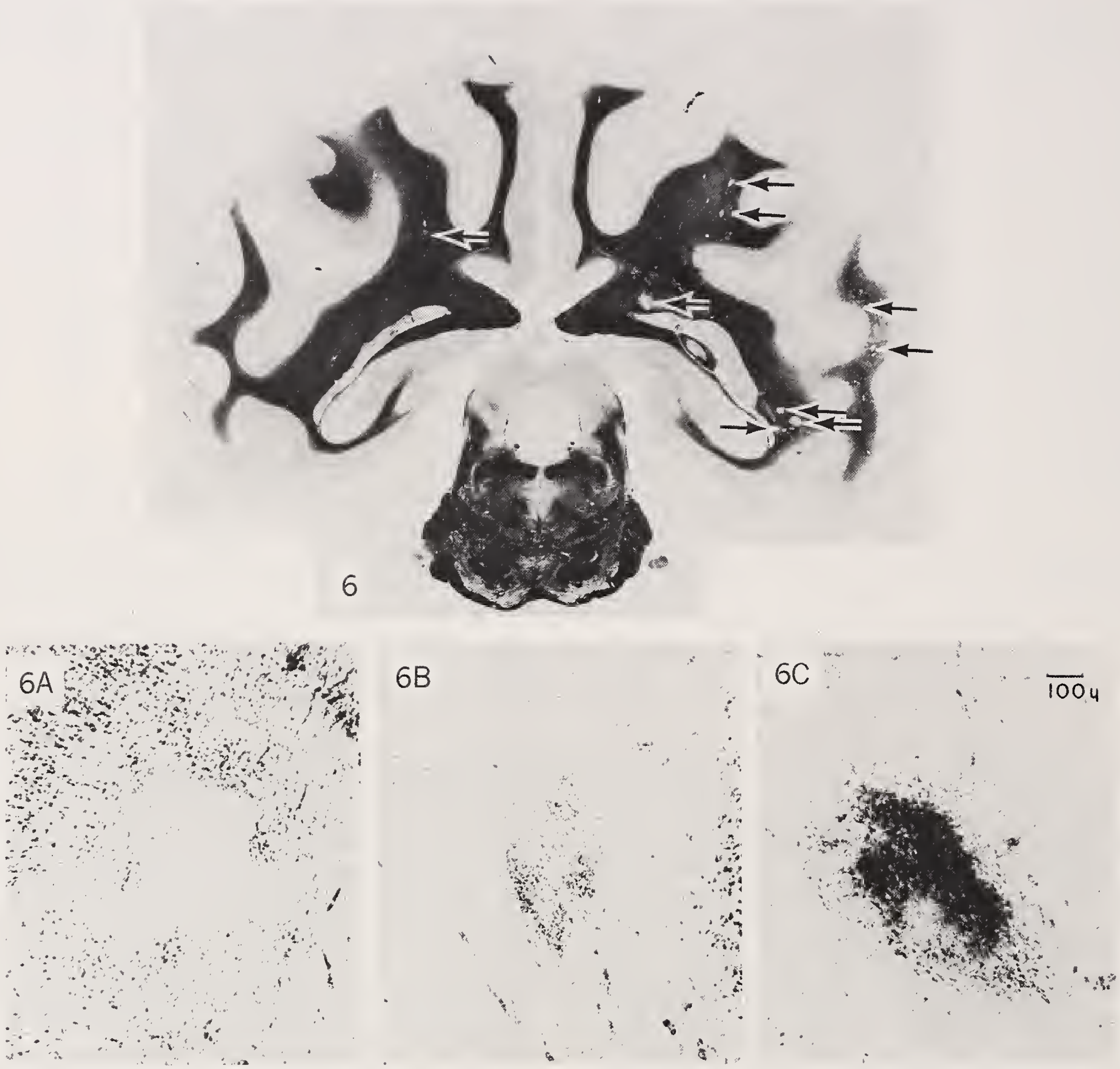
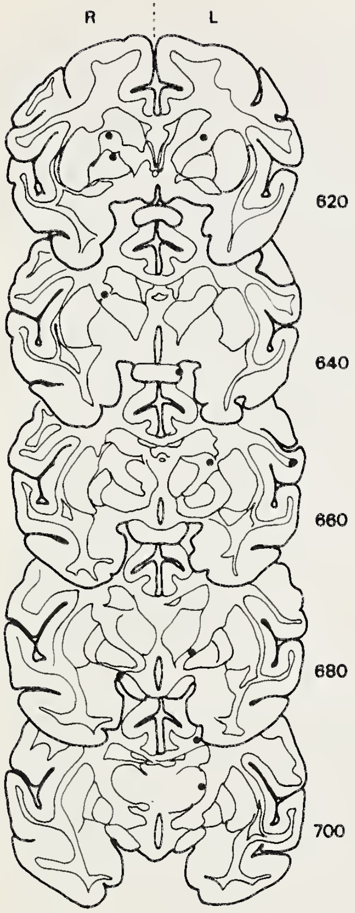


Figure 6.—*Top:* Coronal section of monkey L-13, killed 26 wk after start of 6,000-rad course, shows punctate areas of necrosis (*arrows*). Myelin. × 1.6. *Bottom:* The individual lesions are found to be of different ages. A) The most recent consist of pale areas with a central core of necrotic brain surrounded by a narrow rim of macrophage response, as illustrated on the *left.* B) Slightly older lesions contain numerous macrophages throughout, as that in the *center.* C) The oldest show mineral deposits, as shown on the *right.* The latter stain for calcium and iron. A, B, C: Nissl. × 100

Text-figure 6.—The wide scatter of the small lesions is best appreciated with whole brain serial sections as indicated in these schematic drawings in which the necrotic areas are indicated by *black dots.* Monkey L-13 was killed 26 wk after the start of the 6,000-rad course.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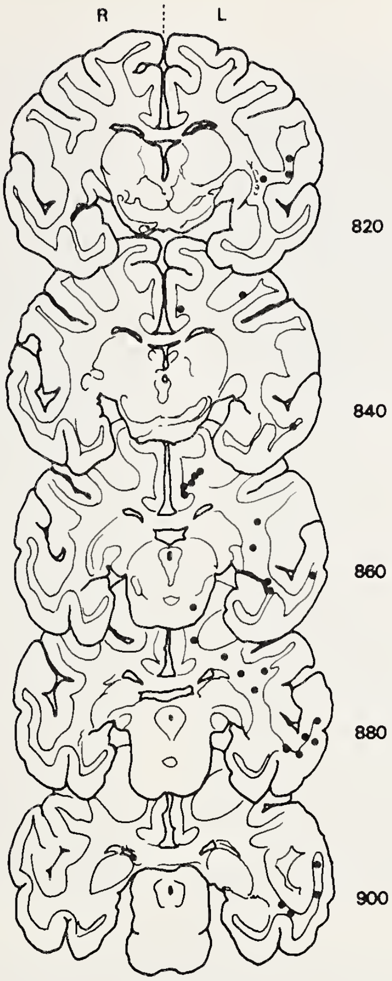
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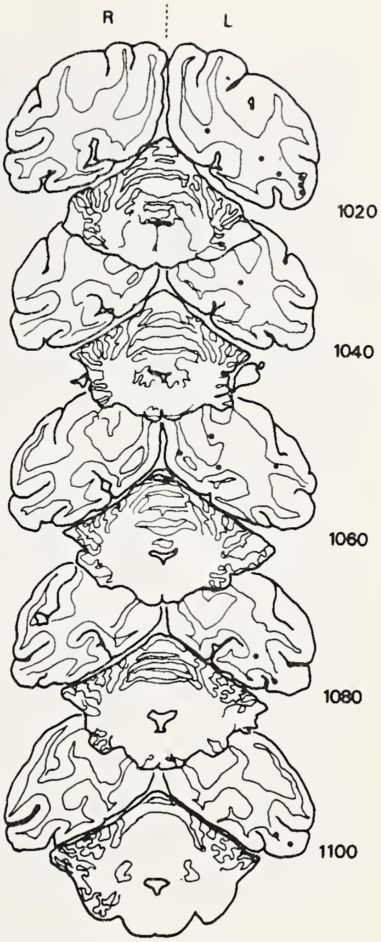
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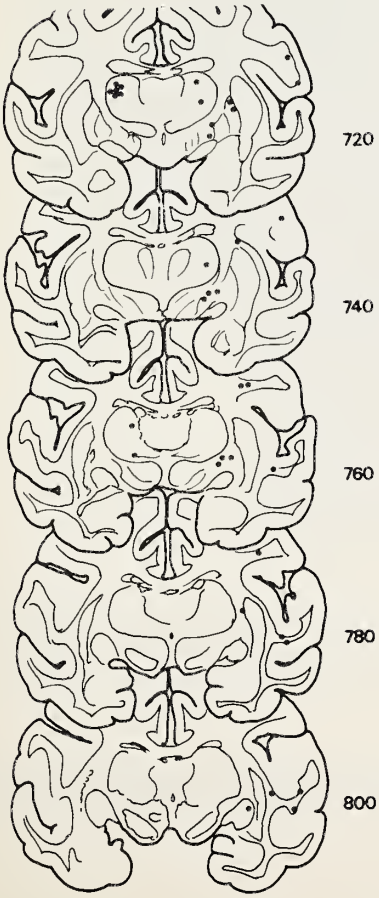
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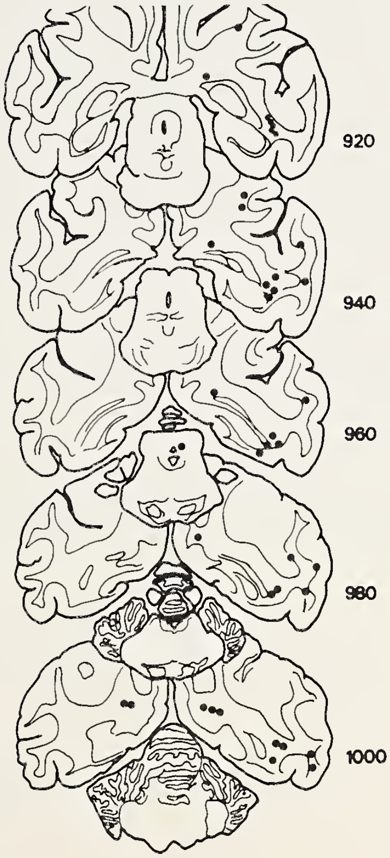
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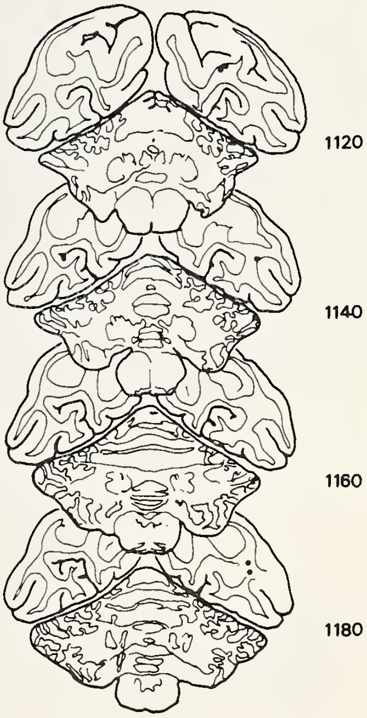
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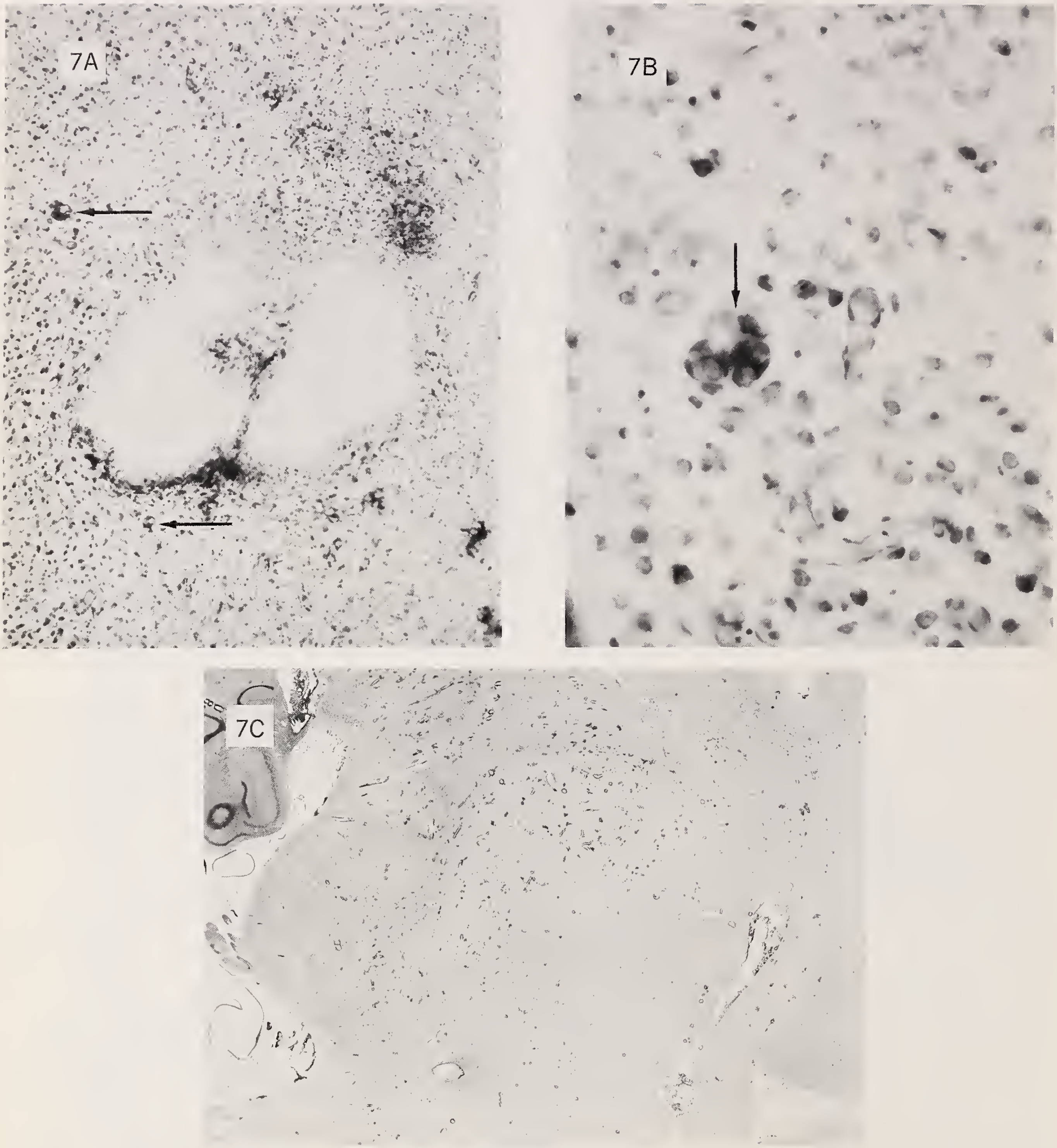

Figure 7.—Monkey L-13, killed 26 wk after start of 6,000-rad course. A) Adjacent to the punctate lesions are occasional grossly altered vascular endothelial cells (*arrows*). Nissl. × 100. B) Under higher magnification are seen the heaped-up endothelial cell nuclei. Nissl. × 400. C) Dilated (telangiectatic) capillaries in the thalamus, subthalamus, internal capsule, and alba. PAS. × 6.8

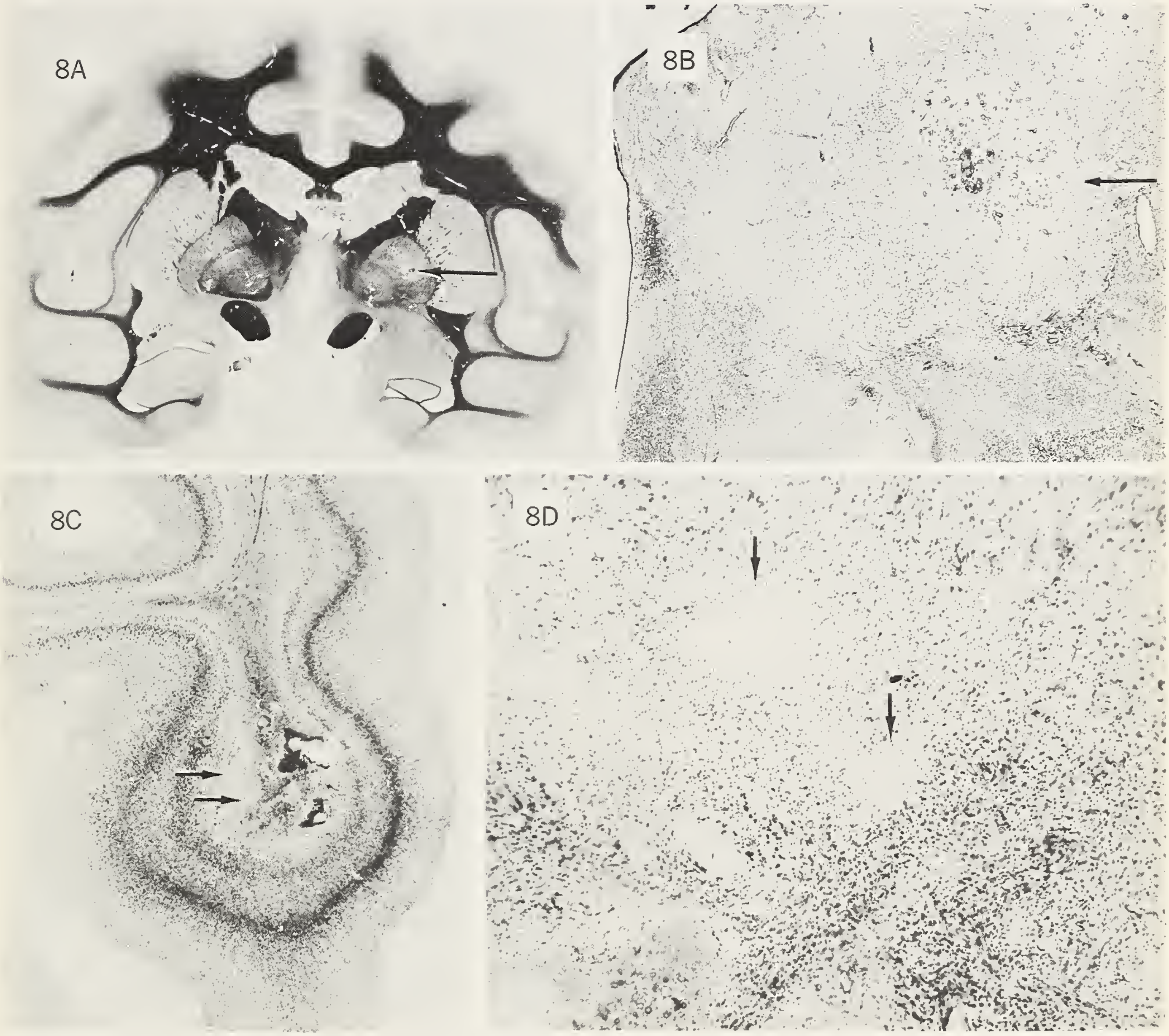

Figure 8.—Monkey L-16, 78 wk after start of 6,000-rad course, showing areas of telangiectasia, mineralization, and fresh necrotic lesions. A) Coronal section with an *arrow* indicating a patch of dilated and ill-formed capillaries in the lateral aspect of the globus pallidus. Myelin. × 1.6. B) Same area. PAS. × 50. C) Temporal cortex, with underlying areas of mineralization along with fresh necrotic lesions (*arrows*). Nissl. × 16. D) Same area. Nissl. × 100

few of these were recent, sustaining the impression of a trend toward repair.

The effects from 8,000 rads at 26 weeks, e.g., L-20 with 64 focal lesions, were similar in kind but greater in degree than those from 6,000 rads. L-22, killed off schedule at 32 weeks, showed 2,089 focal lesions throughout the white and gray matter of the brain, and 115 in the brain stem. An example of focal edema in this monkey is illustrated in figure 9. L-23, killed at 52 weeks, demonstrated a confluence of necrotic lesions in the swollen cerebral hemispheres including thalamus and basal ganglia but fewer focal lesions in the brain stem than were found in monkey L-22, incapacitated at 32 weeks. L-21, killed at 78 weeks, also had massive brain destruction from confluent necrotic lesions in the cerebral hemispheres, with still fewer lesions in the brain stem than in L-23. The loss in brain substance was reflected in the atrophy of the gyri and marked enlargement of the lateral, third, and fourth ventricles and the aqueduct. These monkeys exposed to 8,000 rads demonstrate, with the passage of time, a consistent, progressive increase in number and confluence of necrotic lesions. Even within this relentless course, the monkey killed at 78 weeks showed a less active and more atrophic process than the monkey killed at 52 weeks.

This third model, in which fractionated exposure to high energy irradiation was used, suggests the following conclusions:

1) The extent of the delayed effects is dose dependent. The effects from 4,000 rads are negligible; from 6,000 rads, considerable; from 8,000 rads, profound.

2) The necrotic lesions from 6,000 rads are small and widely scattered, with a predilection for the forebrain white matter but not excluding the central gray matter and brain stem. The possibility of focal as well as general clinical phenomena is inherent in this kind of brain damage.

3) The time course is important. Not only is there a delay of weeks or months before overt tissue breakdown but the individual lesions evidently begin their cycle at different times, and it is probable that their overall influence extends over a protracted period. Although a trend toward recession in numbers of fresh necrotic lesions is apparent, adequate repair may be delayed or may never occur. At 1 year, the effects from 6,000 rads include considerable mineralization of the necrotic lesions but a far greater extent of telangiectasia than is found at 6 months.

4) At one phase in the developing areas of necrosis, multiple minute breaks may appear in the blood–brain barrier. When sufficiently numerous, these can cause diffuse brain swelling, reflected by papilledema.

5) The lesions from 8,000 rads at 6 months are not strikingly different from those following 6,000 rads; however, by 12 months, the extensive and coalescing lesions from the former produce gross brain destruction.

DISCUSSION

Delayed radionecrosis in the brain following therapeutic efforts in man have been reported periodically over the last 4 decades (*7–11*). These effects, after intervals of a few months to several years, have resulted from single or multiple exposures with orthovoltage or supervoltage radiation. Three dramatic cases, recently reported, describe how the delayed effects can simulate a tumor, i.e., an expanding mass, in the brain or spinal cord, respectively (*12–14*). In animal investigations, the monkey brain has been exposed in whole or in part to ionizing irradiation from a variety of sources, with the accumulation of precise information on delayed breakdown in tissue of the central nervous system (*15–18*). However, to our knowledge, no previous studies have involved the exposure of the whole brain alone, in fractionated doses, to supervoltage radiation.

The principal advantage in simulating a therapeutic radiation regimen in a monkey model is to observe the effects in a brain uncomplicated by preexisting pathology, e.g., that resulting from a neoplasm, surgical trauma, or chemotherapy. The dosage of 6,000 rads in 6 weeks is the same as that given in human therapeutic efforts, with two exceptions: 1) The source of the high energy therapy is usually cobalt 60 or clinical linear accelerators operating at 4–6 MeV, whereas in our animal model, a 20-MeV linear accelerator was used. 2) It is the general therapeutic practice to expose alternate sides of the brain on the same or alternate days, whereas in the animal model, a single side was exposed throughout the course of radiation. The small size of the monkey head, compared with that of man, and the height of the energy used prompted our colleagues at the Armed Forces Radiobiology Research Institute to advise that the "fall-off" in absorption through the monkey brain was so small that the exposure

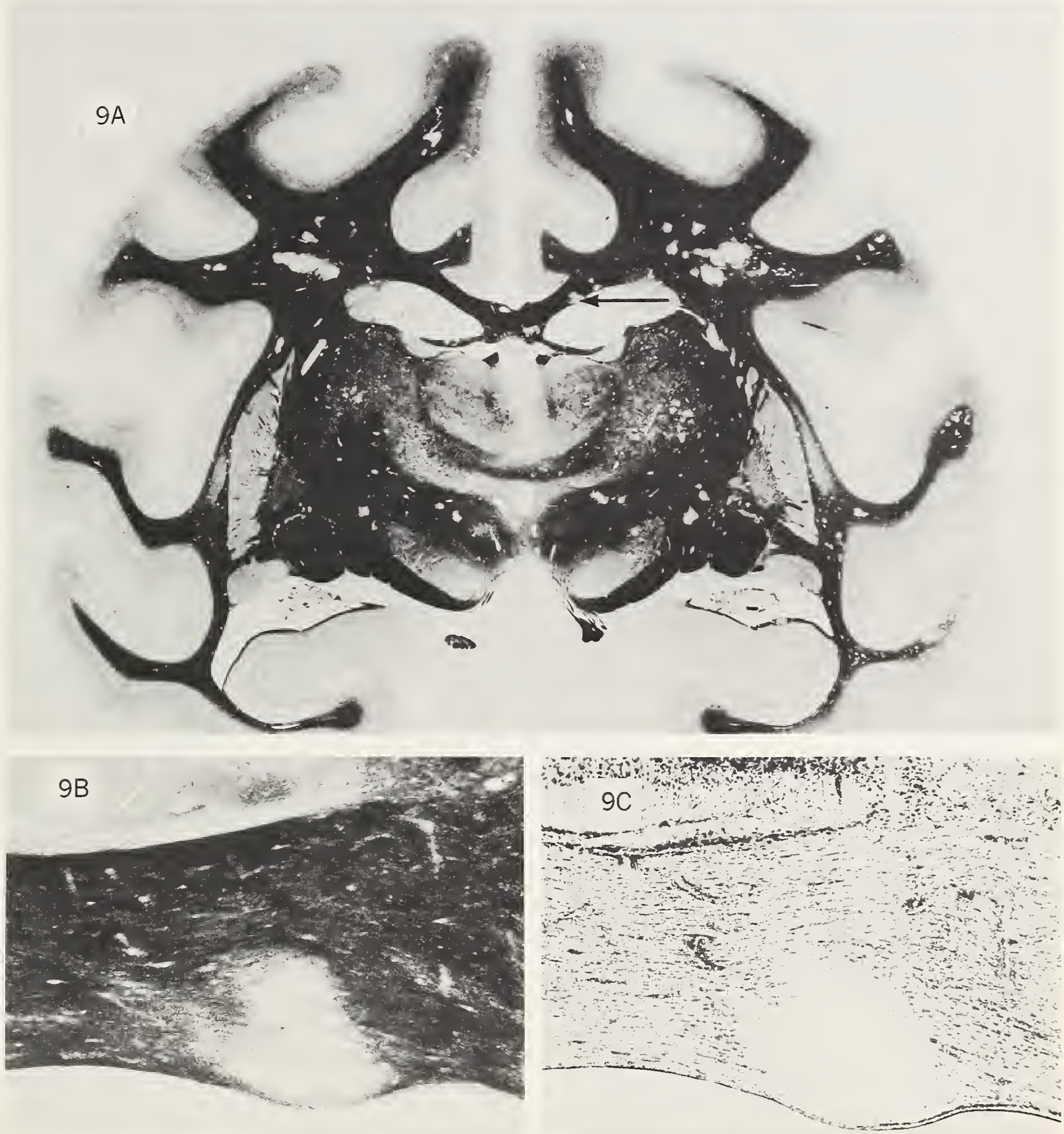

FIGURE 9.—Monkey L-22, killed 32 wk after start of 8,000-rad course. A) Coronal section showing wide distribution of focal necrotic lesions, some of which are beginning to coalesce. Myelin. × 1.6. B, C) Area indicated by the *arrow* in (A) to be accompanied by focal vasogenic edema. At the periphery of the necrotic lesion, adjacent sections stained for myelin and with Nissl show that fibers and cells are separated or pushed aside, evidently the result of a local break in the blood–brain barrier. × 50

of alternate sides was not necessary. The principal disadvantage of the animal model is that inherent in any species other than man when one wishes to interpret results in human terms. Furthermore, in irradiating the normal brain of the monkey, we are gaining no insight into the biologic variations in the human host whose brain is reacting to a growing neoplasm, surgical trauma, or chemical agents. Undoubtedly, these influence the effect of irradiation on the altered brain tissue. Nevertheless, we believe it is scientifically imperative to establish a base line by studying the effects on a normal brain before proceeding to more complicated determinations. We have a current series of 21 adult monkeys, 8–12 kg, that have been exposed to 6,000 rads of supervoltage radiation in a 6-week course, alternate sides of the whole brain being exposed on alternate days. These are being killed, in sets of 3 and at sequential intervals following the beginning of the radiation course, to provide a more precise understanding of the development and degree of recession of the lesions produced by this mode of irradiation. We hope that this kind of information will be useful in the planning of therapeutic efforts in man, with attention to "risk-benefit" factors, and in the interpretation of fresh neurologic and pathologic findings after therapy.

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Neuropathology of Tissues From Patients Treated by the Brain Tumor Study Group[1,2]

M. S. Mahaley, Jr.,[3] **F. Stephen Vogel,**[4] **Peter Burger,**[4] *and* **Nitya R. Ghatak**[5,6]

ABSTRACT—The histopathologic diagnoses in 718 brain tumor patients entered in the Brain Tumor Study Group were reviewed, as well as those for 53 of these patients who were autopsied later. This review documented instances of progression of histologic anaplasia. Of particular interest in the autopsied cases were several instances of extensive necrosis in white matter distant from persisting glioma following chemotherapy and radiotherapy. This observation suggested the presence of a structural and/or metabolic alteration in the diseased hemisphere that perhaps makes it more susceptible to further alterations secondary to the adjunctive therapy.—Natl Cancer Inst Monogr 46: 77–82, 1977.

Over the last several years, we examined slides from the surgical specimens and from a limited number of autopsies on patients entered into the Brain Tumor Study Group (BTSG) from the institutions listed in table 1. We served as a neuropathology review committee to give uniformity to the pathologic identification of surgical specimens and to evaluate pathologic changes occurring in tissue taken at operation and that removed later at postmortem examination. Although the conclusions reached in our reviews have been made available to the participating institutions, we never intended to impose our tissue classification on the referring neuropathologist or to dictate to the clinician the type or duration of treatment to be used. In fact, we did not know what treatment any patient received until after the neuropathologic review was completed, and our efforts were directed to providing uniformity in the neuropathologic diagnosis of the surgical specimen and to evaluating the postmortem changes in the neoplasms and surrounding brain tissues.

The material in this report was taken from two consecutive phase III studies on treatment of anaplastic gliomas by the BTSG. All the cases entered in this study were adults with supratentorial brain tumors. The treatment protocols for these phase III studies are shown in text-figures 1 and 2. Within 3 weeks after surgical resection of as much tumor tissue as possible, each patient was placed randomly into one of four categories of treatment as outlined in the text-figures. Cobalt therapy consisted of 6,000 rads to the whole head for a 6-week course. The dosage of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) was 80 mg/m^2/day iv × 3, repeated every 8 weeks; 220 mg 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU)/m^2 was given orally once and repeated every 8 weeks. We followed the patient's progress until her death for a final determination of the relationship between postoperative treatment and survival. It was expected that the neuropathologic review of these cases might permit some further definition of the relationship between tumor type and survival time and the identification of any particular sensitivities of certain brain tumor types to any one of the several treatments employed. A total of 715 surgical specimens was reviewed from 1969 through 1975. Five hundred twenty-three of these patients have succumbed, and of this number, 110 have been autopsied. Histologic sections from 53 of the autopsy cases were available to our committee for neuropathologic review, and autopsy protocols were received on 31 of these. In 8 cases, the brain only was available for examination at the time of autopsy.

Table 2 summarizes the pathologic diagnoses of the surgical specimens as evaluated by our committee. More than 75% were judged to be glioblastoma multiforme, whereas 92% of all cases were characterized as anaplastic gliomas of astrocytic, ependymal, or oligodendroglial derivation.

Table 3 defines the relationship between pathologic diagnosis, treatment methods, and survival of glioma patients. Any one of the several treatment protocols improved survival time of glioma

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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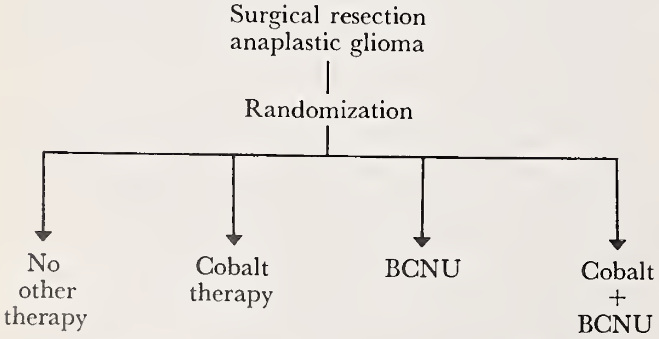
[6] All authors served on the Neuropathology Review Committee of the Brain Tumor Study Group.

TABLE 1.—*BTSG participants*

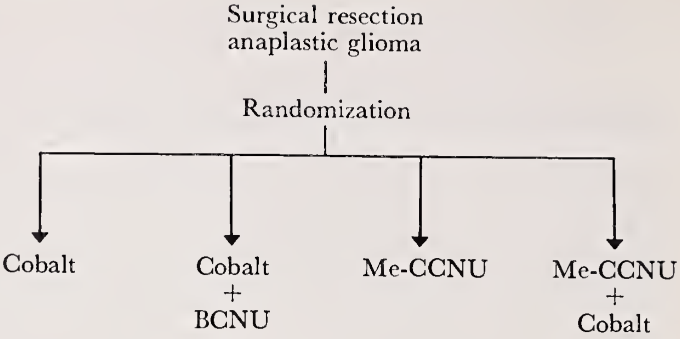
Baltimore Cancer Research Center
Bowman Gray School of Medicine
Duke University Medical Center
Indiana University School of Medicine
New York University Medical Center
Ohio State University Hospital
Sloan-Kettering Memorial Hospital
St. Louis University School of Medicine
University of California at Los Angeles
University of California at San Francisco
University of Pavia (Italy)
University of Kentucky Medical Center
University of Tennessee

patients with glioblastoma multiforme compared with that of control patients treated with surgical resection alone. It appears that the most effective of these treatments is the combination of whole-head radiation therapy and systemic BCNU chemotherapy. In this study, the number of patients with anaplastic gliomas other than glioblastoma is small. However, the data suggest that patients with other forms of anaplastic gliomas survive longer in the treated and control groups. For example, the patients with anaplastic astrocytomas treated with cobalt therapy plus BCNU chemotherapy survived an average of 51.9 weeks compared with 41.4 weeks for similarly treated patients with glioblastoma. The same is noted in patients with benign gliomas. These findings reaffirm that histologic diagnosis is one of the determinants of survival time. They also suggest that within a given histologic category, such as glioblastoma multiforme, radiation alone or in combination with chemotherapy prolongs survival.

Because of the sampling problems inherent in small biopsies, caution must be exercised in comparing the findings in such specimens with the pathologic changes encountered at autopsy. Nevertheless, it was our impression that the histologic characteristics of the neoplasm in the postmortem material either were similar to those in the surgical specimens or, more commonly, evidenced greater degrees of anaplasia associated with increased endothelial proliferation and necrosis (table 4, figs. 1, 2). Thus in one-fifth of the glioblastomas, the postmortem specimens had more abundant areas of necrosis than the earlier surgical specimens. In the autopsy material, most of the sections from anaplastic astrocytomas also showed increased evidence of anaplasia, and the same was true of 1 case of benign astrocytoma. The 1 patient with oligodendroglioma succumbed within 1 week after operation and, as expected, there were no changes in histologic pattern between biopsy and autopsy.



TEXT-FIGURE 1.—Scheme of randomization in 69–01 phase III study of BTSG.



TEXT-FIGURE 2.—Scheme of randomization in 72–01 phase III study of BTSG.

For 31 of the 51 autopsy cases, protocols were available for determination of the causes of death (table 5). Appreciable pneumonia was seen in 14; in most instances, recurrent tumor was also a major contributory factor. Six patients had had pulmonary embolization of sufficient size to have made a major contribution to their death.

We searched for any evidence of histologic alterations in areas of brain that did not contain

TABLE 2.—*Histopathologic diagnoses in 718 surgical specimens*

Diagnosis	No. of cases	Percent of total
Glioblastoma multiforme	548	76.3
Anaplastic astrocytoma	72	10.0
Anaplastic ependymoma	28	4.0
Anaplastic oligodendroglioma	3	0.4
Mixed anaplastic glioma	9	1.3
Astrocytoma	35	5.0
Ependymoma	4	0.5
Oligodendroglioma	10	1.4
Benign glioma, unclassified	3	0.4
Neuroblastoma	1	0.1
Metastatic carcinoma	1	0.1
Lymphoma	1	0.1
No diagnosis	3	0.4
Total	718	100.0

TABLE 3.—*Valid Study Group: average survival, wk*[a]

Diagnosis	Control	Treatment				
		Me-CCNU	BCNU	Cobalt	Me-CCNU + cobalt	BCNU + cobalt
Glioblastoma multiforme	14.3 (0/26)	24.3 (10/47)	23.8 (0/44)	33.9 (12/109)	33.6 (9/44)	41.4 (15/106)
Anaplastic astrocytoma	28.7 (0/3)	70.2 (3/5)	9.8 (0/4)	68.4 (2/10)	36.1 (2/7)	51.9 (7/15)
Anaplastic ependymoma	21 (0/1)	19.2 (1/4)	37.3 (0/3)	43.2 (1/5)	14 (0/1)	57.2 (3/9)
Anaplastic oligodendroglioma		30 (1/1)		56 (0/1)		
Mixed anaplastic glioma	22 (0/1)			94.7 (1/3)		26.2 (0/4)
Astrocytoma	10.5 (0/2)	64.5 (1/2)	71 (1/3)	32 (1/2)		68 (3/9)
Ependymoma				38 (1/2)		227 (1/1)
Oligodendroglioma		42 (1/1)		131 (1/2)		53 (1/1)
Mixed benign glioma, unclassifed					11 (1/1)	

[a] *Numbers in parentheses* are: numerator=living cases; denominator=total cases.

tumor. Such examinations were possible in 32 cases, and the findings are shown in table 6. The types of alterations included infarction of cerebral tissue, spongiform changes, necrosis within the white matter, hyalinization and fibrinoid changes within the walls of blood vessels, perivascular inflammatory cell reactions, mineralization of the tissues, and loss of neurons. Table 7 illustrates the higher frequency of these findings in patients with longer survival times. The most striking changes occurred in a patient with glioblastoma who survived 101 weeks; he received systemic chemotherapy in conjunction with irradiation. A coronal section of this brain showed little residual tumor but exhibited extensive necrosis in the white matter of the left cerebral hemisphere, lateralized to the side of the neoplasm, and extending well beyond the depth of the neoplastic infiltration (fig. 3). This observation suggests possible chemotherapy-induced potentiation of radiation necrosis in a hemisphere that harbors a neoplasm.

TABLE 4.—*Correlation of surgical and postmortem diagnoses*[a]

Surgical pathologic diagnosis	Autopsy pathologic diagnosis, cases			Scant tumor
	No change	↑ Anaplastic	↑ Necrosis	
Glioblastoma (37)	21	7	7	2
Anaplastic astrocytoma (7)	1	6		
Anaplastic ependymoma (2)	1	1		
Anaplastic glioma, unclassified (2)		2		
Astrocytoma (1)		1		
Oligodendroglioma (1)	1			

[a] *Numbers in parentheses* indicate number of cases with that tumor.

TABLE 5.—*Contributing causes of death (31 autopsy protocols reviewed)*

Cause	No.
Recurrent tumor only	9
Pneumonia	14
Pulmonary emboli	6
Wound infection	2

TABLE 6.—*Incidence of histologic alterations in 32 cases in areas of brain distant from neoplasm*[a]

Treatment categories	No. of cases with alterations
Cobalt (5)	1 Glioblastoma
Cobalt + BCNU (11)	4 Glioblastomas 1 Astrocytoma→GBM
Cobalt+Me-CCNU (2)	2 Glioblastomas
BCNU (7)	3 Glioblastomas
Me-CCNU (3)	None
No therapy (4)	None

[a] *Numbers in parentheses* indicate number of cases.

TABLE 7.—*Relationship of length of survival to occurrence of alterations in brain distant from neoplasm in 28 treated cases*

Survival, wk	No injury	Injury
0–32	12	3 (20%)
>32–52	3	5 (62%)
>52	2	3 (60%)

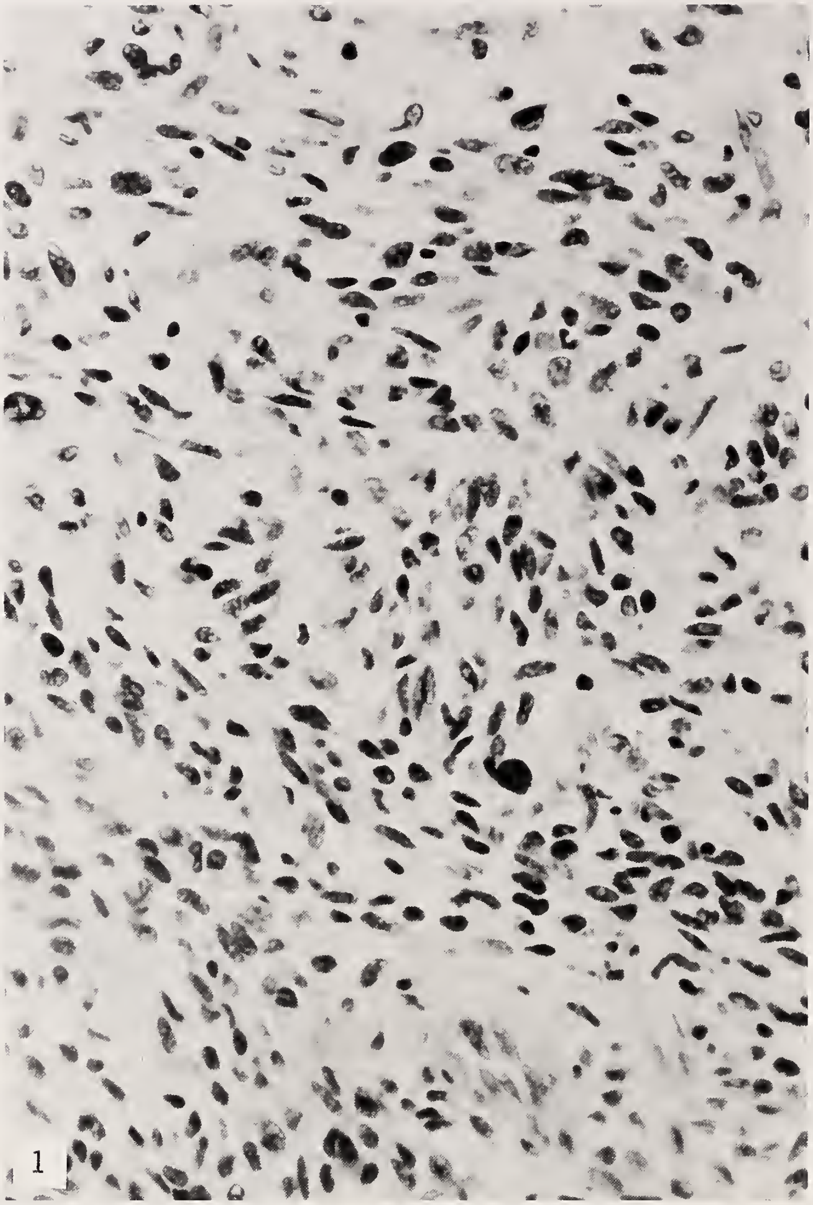


FIGURE 1.—Surgical specimen of a mixed anaplastic glioma, demonstrating moderate cell density with fairly uniform nuclear patterns. × 400

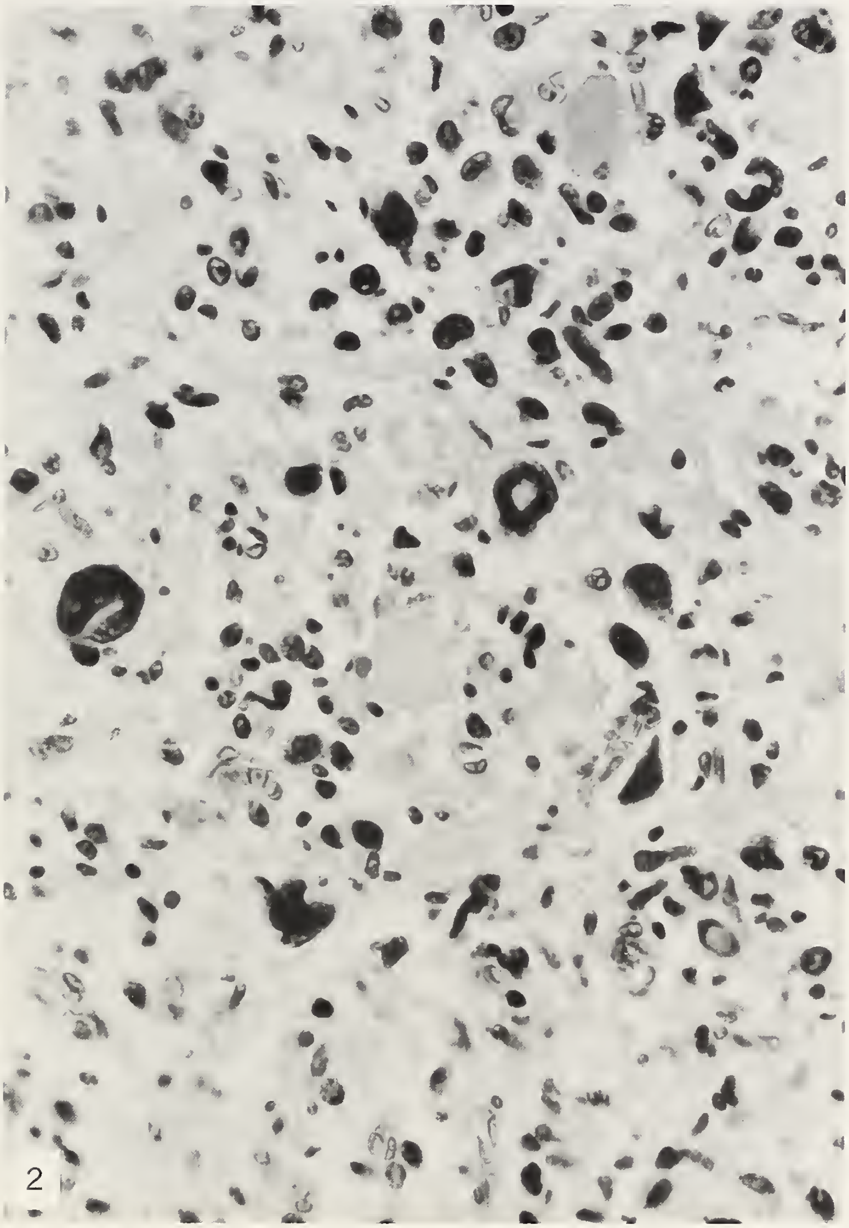


FIGURE 2.—Autopsy specimen on same case as figure 1, showing more bizarre nuclear and cellular pleomorphism 65 wk after operation and treatment with cobalt therapy plus BCNU. × 400

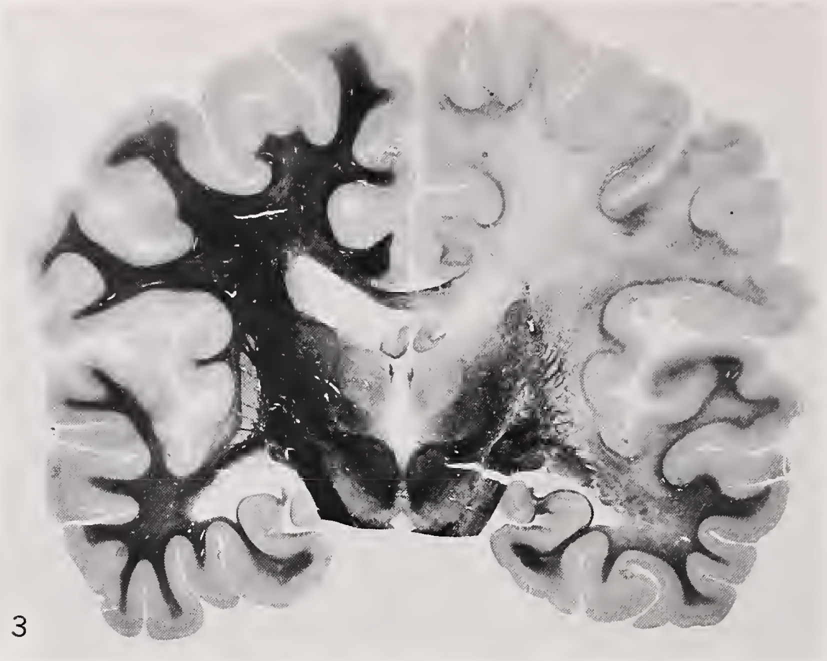


Figure 3.—Whole-mount coronal section of patient treated with 6,000 rads whole-head, cobalt therapy, and 9 bimonthly BCNU injections. He survived 101 wk.

This review reaffirms a direct relationship between the histologic characteristics of a glioma and the survival time, particularly in the frequency of neoplastic transformation in tumor tissue between the time of surgical resection and postmortem examination. In each instance, the transformation was characterized by an increased degree of anaplasia. In a single case, we found extensive necrosis of the white matter in the tumor-bearing hemisphere almost 2 years following combined therapy with irradiation and BCNU.

Pathologic Tumor Type and Response to Treatment[1]

Richard L. Davis[2]

ABSTRACT—The general biology of the common primary brain tumors is reviewed, as are the results of surgery, radiation therapy, and chemotherapy for these neoplasms. The problems of classification, particularly of the astrocytomas, are noted, with the resultant difficulties in comparing different series. The development and consistent application of a widely acceptable nomenclature for brain tumors would be useful in the comparison of various therapies and for ease in communication. Further, a better general understanding of the biology of the various brain tumors is desirable. Reliable data relative to old or new forms of therapy seem to require national or regional studies in view of the relative rarity of some of these tumors. Except for medulloblastoma, the best results of therapy thus far are only slightly encouraging. We must also be aware that some of our treatments may do as much harm to some patients as their tumors; therefore, we must all strive for a high autopsy rate so that we can monitor our therapy and recognize unexpected complications as quickly as possible.—Natl Cancer Inst Monogr 46: 83–87, 1977.

It is unusual for a pathologist to be discussing the role of a pathologic tumor type in the response to different treatment modalities, as this subject is more frequently presented by the neurosurgeon, the radiation therapist, or the chemotherapist. Furthermore, it would be presumptuous for a pathologist to attempt to discuss surgical approaches, theoretical or actual, specifics of radiation therapy, or the theory and practice of chemotherapy, some of which have been recently and capably reviewed (*1–8*). The immunotherapy of human brain tumors is so new that its potentialities and ultimate role in treatment remain entirely speculative (*9*). None the less, though a neuropathologist cannot hope to match operative strategies, rads or ports, or cytolytic agents, he can add a facet of understanding in attaining our common goal: the cure or at least the amelioration of brain tumors.

Since we are involved in a scientific venture attempting to learn what therapy or combinations of therapy most effectively destroy or slow the growth of brain tumors, we must have as certain a specific diagnosis as is possible. Although a pathologist cannot help but speak from a biased viewpoint, and though we have great respect for our neurosurgical and neuroradiologic confrères, it seems evident that a diagnosis without biopsy evidence or autopsy proof must be viewed with caution. This is particularly true when a mode of therapy is used on such a patient that in other proved cases does not give good results but in these results in a most unusual long survival. Though treatment should not be denied patients with, e.g., presumed brain stem gliomas or deep cerebral lesions, it is only logical that such cases should be considered as "presumptive" and not as "proved" in our evaluation of results.

Furthermore, to best evaluate our therapy, we should have complete autopsies when our patients die, whether "cured" or not. Only in this way can we know (to the extent possible by this means of study) what happened. Did the tumor continue to grow? Did edema from our therapy cause death? Did some secondary factor or factors lead to our patient's demise? The discovery by Price and Jamieson (*10*) and Rubinstein et al. (*11*) of a peculiar leukoencephalopathy apparently associated with combined radiation therapy and intrathecal methotrexate in children with leukemia and lymphoma of the central nervous system (CNS) is a good example of the latter. We have seen a similar case (unpublished observations) recently in a youngster treated for CNS acute lymphocytic leukemia with intrathecal methotrexate and neuraxis radiation. He developed severe brain stem symptomatology and, at autopsy, showed extensive destructive lesions mainly in the pons, with little involvement of the cerebrum. The lesions seen in this patient are identical in location and morphology to those described by Rubinstein et al. (*11*) and seem distinct from those described by Shapiro et al. (*12*), after intraventricular administration of methotrexate.

We have been surprised at the higher than expected incidence of seeding of the spinal subarachnoid space by tumor in our patients with glioblastoma multiforme. We noted this, of course, since we began routinely to examine the spinal cord, an unfortunately uncommon part of the routine autopsy. In the last 6 years, we have seen 20 glioblastomas at autopsy, 19 in the cerebrum and 1 in the midbrain. In 3 of these, extensive seeding of the spinal subarachnoid space was found, and in 1, a midbrain neoplasm, there was additionally seeding upward, ventricular tumor being present in the anterior lateral ventricles (unpublished observations). Though seeding by neuroepithelial neoplasms of the subarachnoid space has been reported (*13, 14*), the frequency in our series is higher than in most others, and the therapeutic implications of this possibility have been ignored except for medulloblastoma and ependymoma. In consultation, we saw a young woman with a parasagittal malignant mixed glioma whose primary lesion seemed under good control. Her demise was due to extensive diffuse involvement of the subarachnoid space by the tumor. Possibly, with increased success controlling the primary lesion, we will be faced more often with problems due to this type of dissemination.

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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ASTROCYTOMA

The amazing inconsistency of nomenclature relative to tumors of the nervous system makes comparison between series extremely difficult. This is particularly true of the astrocytic neoplasms, which have a large array of descriptive options that are used inconsistently even at the same institution. Therefore, at least some of the comparisons that follow must be interpreted carefully.

JUVENILE PILOCYTIC ASTROCYTOMA

Juvenile pilocytic astrocytoma, most commonly found in the cerebellum of children, seems clearly to be treated best neurosurgically in that site; additional radiation therapy offers little in prolonged survival. The recent series of Gilles et al. (*15*) with 94% 10-year survivors supports Bouchard's earlier evaluation (*16*) that postoperative radiation therapy resulted in no statistically significant advantage over surgical treatment alone, even in those lesions incompletely excised.

Therefore, the recent reports by Sheline (*8*) and Marsa et al. (*17*) of good results with radiation therapy for such tumors must be considered in the knowledge of the slow growth rate and long survival of patients with these neoplasms, even with incomplete surgical resection. However, good follow-up to autopsy in these patients has not been accomplished in any published series, and Sheline (*8*) suggests that total removal was not possible in their cases because of involvement of vital structures. The same tumor in the anterior optic pathways is comparably cured by surgery [(*18*); Yanoff M, Davis RL, Zimmerman LE: Manuscript in preparation], and some even contend that this is not needed (*19*). In the more posterior optic nerve and chiasmatic sites, as well as in the adjacent hypothalamus, this tumor cannot be as effectively approached surgically, and in these circumstances radiation therapy may be efficacious (*17*). As in the other sites, one must bear in mind when evaluating these results that these neoplasms most often grow slowly and even in the hypothalamus may have a prolonged course.

Brain stem juvenile pilocytic astrocytomas also occur, and one series (*17*) reports a 20% incidence of this neoplasm among the brain stem gliomas. Marsa et al. (*17*) have, in fact, suggested that the long-term survivors among patients with irradiated brain stem tumors may represent the natural evolution of the juvenile pilocytic astrocytomas in that site. Since many brain stem tumors are not biopsied, this question may long be unanswered unless follow-up to autopsy is obtained.

Cerebral hemispheric juvenile pilocytic astrocytomas are rare but are asserted to be biologically identical to those in the cerebellum (*20*). It may be that some long-time survivors of "low grade" cerebral astrocytomas are due to their being this type.

Chemotherapeutic agents seem to be untried in these tumors as a group, so data relative to their effectiveness are not available. They are probably not indicated except for recurrent tumor or radiation-resistant lesions in inaccessible sites.

INFILTRATING ASTROCYTOMAS AND GLIOBLASTOMA MULTIFORME

The infiltrating astrocytomas, be they fibrillary, fibrous, protoplasmic, pilocytic, gemistocytic, or diffusum, are the most common glial tumors, except for glioblastoma multiforme of the cerebrum and brain stem (*20*). They are most common in young adults, except that the brain stem tumors also are seen in children (*20*). In the cerebrum of young adults 18–38 years of age, they show transitions to glioblastoma less than half as frequently as in older adults (*21*). Their infiltrative nature defies surgical extirpation because most often even the pathologist cannot be certain of the periphery of the neoplasm. A cytologic continuum occurs with "benign" but nonetheless infiltrative tumors on one end and the malignant glioblastoma[3] on the other (*20, 21*).

The "grading" of astrocytomas was popularized by Kernohan and Sayre (*21*) and was based mainly on the cytology of the tumor cells. In their grade 1 lesions, they included not only cytologically nonanaplastic infiltrative lesions of the cerebrum but also the cystic astrocytomas of the cerebellum, referred to above as juvenile pilocytic astrocytomas. As they defined grade 2 lesions, anaplasia was a significant feature, as was increased cellularity and at least some vascular endothelial proliferation. Grades 3 and 4, which were parenthetically termed "glioblastoma multiforme," had marked anaplasia, endothelial and adventitial proliferation, and, commonly, necrosis. Grade 4 was described as an extremely bizarre form of glioblastoma. However, as this system has been used by others (*3, 22*), it seems that lesions that are anaplastic or malignant astrocytomas have been called grade 3, rather than grade 2, and that at least sometimes only glioblastoma multiforme has been termed a grade 4 astrocytoma. This distinction in usage may explain some of the variations in results when comparing different series.

The much lower incidence of transition to glioblastoma in the younger adults (i.e., less than 40 years of age) does suggest that these astrocytomas in this age group may be more uniform histologically than in the older age groups. In the latter, the problem of histologic sampling of a lower grade lesion in an individual with a glioblastoma is well known.

The difficulty of comparing various series and results of therapy is probably most obvious with the infiltrating astrocytomas. Even the grading system does not seem to be consistently applied and must certainly be carefully interpreted, for in a 25- or 30-year-old individual, a grade 2 astrocytoma might well be compatible with a natural life expectancy of several years, whereas in a 60-year-old, the chances of the tumor being a glioblastoma elsewhere at the time of biopsy is probably well over 50%.

Table 1 shows some of the results for low-grade cerebral astrocytomas from a few selected series (*16, 23–25*). With the exception of the Uihlein series (*24*), radiation therapy seems to add significantly to survival and is certainly the most accepted treatment in addition to surgery. This table illustrates the problem of terminology, for in Levy and Elvidge's report (*23*), the tumors were classified by the Penfield classification, but Bouchard (*16*), reporting on 87 of the 89 cases, used grades 1 and 2. Further, Uihlein's data are based on results in the patients who could be traced. The percentages based on the original sample (though this is the worst interpretation) give results more comparable to the other series.

TABLE 1.—*Cerebral astrocytomas*

References	Tumor type	No. of cases	5-yr survivors, %		
			Surgery	Surgery + radiation	All
Levy and Elvidge (*23*)	Piloid	38			37
	Diffusum	22			27
	Gemistocytic	24			25
	Borderline	5			40
Total		89			32
Bouchard (*16*)	Grades 1, 2	87 105	26	36 49	31
Uihlein et al. (*24*)	Grades 1, 2	83	64.7 (33)[b]	53.6 (30)[b]	57.7[a] (31.3)[b]
Stage and Stein (*22*)	Grade 2	38	20	32	

[a] This value is 62.5% if >3,500 roentgens (R) are given.
[b] Value is based on all cases.

Though it seems reasonably clear that radiation therapy in addition to surgery is probably beneficial for patients with these low grade astrocytomas, it is unclear what chemotherapy has to offer in addition. Chemotherapeutic agents are used for malignant astrocytomas, and whether others consider these low grade lesions malignant (even though they are infiltrating) is difficult to determine from the published reports.

The long-term results of treatment of malignant astrocytoma and glioblastoma are exceedingly poor (*16, 20–22, 24, 25*). Even radiation therapy adds little to long-term survival (*22–24*). This is as high as 54 and 41% in one series (*22*) for grade 3 and grade 4 tumors, respectively. Perhaps of more significance, radiation and the various chemotherapeutic agents may increase the quality of the time left for the patient (*22*). If radiation therapy is used, adequate fields are obviously important; in this regard, the malignant astrocytoma and glioblastoma may actually infiltrate less widely than some of the less anaplastic astrocytomas (*21*). It seems evident that we have a long way to go toward effective therapy for these tumors. However, it may well be that, as Posner and Shapiro editorialized recently (*26*), "if we adopt . . . [the oncologists treating leukemia] aggressive approach to the treatment of CNS tumors and also persevere, long-term survivals of good quality are in the offing."

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[3] Oligodendrogliomas and ependymomas may also show transitions to glioblastoma.

EPENDYMOMA

Ependymomas are rare tumors of the nervous system and are most common in children in the region of the fourth ventricle (*20, 21*). Though they have a reputation for seeding via cerebrospinal fluid pathways, studies by Bouchard and Peirce (*27*) and Shuman et al. (*28*) cast doubt on this except for the very cellular cytologically malignant types. Because of the usually inaccessible sites of these neoplasms, surgery alone is most often not curative. However, radiation therapy in adequate amounts, 4,500 R or more, gives reasonably good 5-year survivals (58–87%) (*27, 29*). Shuman et al. (*28*) have suggested that "Collin's law" may apply to these tumors, so that the apparently "good" 5-year results may not in fact mean cures. Further, it would seem that total neuraxis radiation should be given to those patients who have cytologically malignant ependymomas.

As in all of the rare tumor types, the additive effects, if any, of chemotherapeutic agents are in doubt. Whether or not they will be helpful for primary treatment or for recurrences awaits future study.

OLIGODENDROGLIOMA

Oligodendrogliomas, like ependymomas, are rare tumors of the CNS and are exceedingly uncommon outside the cerebrum (*20, 21*). They have a variable course and are thus difficult to evaluate relative to therapy (*3*). The studies of Sheline (*8*) and Bouchard and Peirce (*27*) suggest that postoperative radiation therapy increases 5-year survival. As with ependymomas, the effects of chemotherapy are not yet known.

MEDULLOBLASTOMA

Medulloblastoma is one of the most common tumors of the CNS in children (*20, 21*). Its propensity to seed the neuraxis via cerebrospinal fluid pathways is well known, and until recently, long-term survivals or cures were exceedingly rare. This is a tumor against which we have made important progress, with a significant percentage of survivors at both the 5- and 10-year landmarks, in some series to 35 and 25%, respectively (*30*). Total neuraxis radiation is necessary for effective therapy and retardation of bone growth is to be an expected complication of such therapy. The use of chemotherapy in addition to radiation is now being tried, but the final results are in the future (*5*).

Several agents have been used for recurrent medulloblastomas after primary radiation therapy. Several have shown activity, but their usefulness has been limited by myelotoxicity (*5*).

In spite of the apparent good results with radiation therapy of medulloblastoma, and the increased survival rate when combined with chemotherapy, we should be cautious in any enthusiasm. Sheline (*3*) noted that "it might be that our large doses (of radiation) only serve to further delay the recurrence." A personal case (unpublished observations), as well as the experience of others (*30*), has suggested that "Collin's law" may apply to this neoplasm, too. In the personal case, a medulloblastoma recurred and killed 19 years after surgery and radiation therapy, just short of the 2*n* plus 9 months of "Collin's law."

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DISCUSSION II

L. Rubinstein: I will use my prerogative as chairman and ask Drs. Caveness and Mahaley to elaborate on the pathogenesis of the lesions that have been described in radiation. One lesion is present in all these brains and is almost pathognomonic, i.e., fibrinoid necrosis of the blood vessel walls accompanied by an extravasation of fibrin into the parenchyma. This phenomenon is so striking that it must somehow reflect selective damage to a specifically vulnerable component of the CNS, that of the capillary blood vessels. One also wonders whether other lesions such as demyelination and parenchymal necrosis may be secondary to capillary damage. This lesion is quite distinct from that described by Price and Jamieson, by us, and by others, in the brains of patients with leukemia who were treated with methotrexate and CNS irradiation. There fibrinoid necrosis, although present to some extent, is not nearly as striking as in simple radiation. We have the impression that in this most recently described type of leukoencephalopathy, the basic mechanism somehow involves the axons primarily.

W. Caveness: Dr. Rubinstein has drawn attention to a most fascinating aspect of this delayed necrosis, but I do not think we know its pathogenesis. We can describe it, but we do not know how it comes about. Haymaker, Zeman, Vogel, and others who have studied these lesions believe an impairment in DNA at the time of drug absorption is present. This may represent a breaking of strands with genetic damage to the subsequent generations of cells. Apparently, from the time of irradiation, the genetic material of the endothelial cells that survive is altered and, subsequently, this may induce a wide range of effects varying from simple sterilization and the dropping out of cells to the most exuberant, almost neoplastic, proliferation. These changes in the endothelial cells take place only after their cycle has reached its next turnover point, which Denekamp of London has suggested is about 120 days. Perhaps when the endothelial cells replicate again, they express themselves in these incompetent fashions, and thereby permit breakdown. This can happen over a period of time because we can speculate that at the time of irradiation, the endothelial cells are at different points in their cycle and, therefore, do not break all at once.

M. Mahaley: I would add that doses a little lower than those which produce striking changes in animals that had no lesions in their brains initially cause lesions in patients who have or have had tumors in their hemispheres. These lesions are restricted to, or chiefly affect, the side of the lesion. The basic mechanism may be vascular, but also there is something unique in the blood vessels, probably in the hemisphere that bears the tumor. Perhaps this predisposition to radiation injury is dependent on changes in the blood–brain barrier or on some influence the neoplasm exerts on the adjacent white matter; these make the diseased brain more sensitive to lower radiation dosages than the normal one. It would be fascinating to determine the changes produced by the lower dosages in the monkey models a month after the ASV had been injected into their cerebral hemispheres.

Rubinstein: This response suggests that the area of edema around a brain tumor may be specifically susceptible. Dr. Caveness, has any work been done on irradiation of monkeys or other animals that had been subjected earlier to one of the classical models of experimental edema, quite aside from the implantation of a tumor? Do we have any data on the effect of irradiation on the edematous brain in experimental animals?

Caveness: I do not know of any such data.

J. Ransohoff: In your monkeys, did the type of fibrinoid necrosis develop to which Dr. Rubinstein was referring? The vascular changes you have shown do not look familiar; hence I am not certain whether this is the same lesion that has been described in humans.

Caveness: The fibrinoid changes are there, but I selected some of the more exotic vascular ones. The range and varieties of the vascular changes might astound you.

Ransohoff: Dr. Rubinstein, were you satisfied? Do the changes you saw look similar to those seen in patients?

Rubinstein: The histologic technique Dr. Caveness uses (Nissl and myelin stains in thick celloidin sections) does not permit an adequate comparison. I accept his assurance that fibrinoid necrosis is present. I think that if his material were processed in paraffin sections and stained with phosphotungstic acid hematoxylin, the phenomenon would be remarkably constant.

Caveness: We have it in our PAS sections.

F. Hochberg: Dr. Caveness, in your second experimental model, you showed at 20 weeks a breakdown of the local blood–brain barrier that had not been seen previously; at the same time, you noted a fall-off in perfusion or in cerebral blood flow of that region. Would you comment on the possibility of giving short-lived chemotherapy at the time when the brain seems most likely to accept that material?

Caveness: One wonders what effect this would have in sealing off the break in the blood–brain barrier. We have not done it, but it is a reasonable approach.

K. Charyulu: Obviously, the radiation damage is accelerated. Years ago, we radiated the spinal cord in rabbits approximately 6 months old, and at the same time radiated the spinal cords of aged rabbits. The dose–time relationships were similar to those we use in humans. We saw damage in the growing but none in the adult animals.

What is the life-span in the monkeys you have irradiated? At what age were they radiated? Can you correlate the damage you observed with the growing CNS? Would you expect to see the same thing in adult monkeys?

Caveness: We irradiated 24-month-old monkeys that weighed between 3 and 3.5 kg. These monkeys begin to menstruate between 18 and 27 weeks, so they are at puberty and comparable to humans 12–13 years of age.

We believe the animals have an increased susceptibility to radiation at both ends of their lifespan. We know that the brain is more sensitive in young children and in senility.

This series is too young to permit any comparison with humans being treated for gliomas. A study organized to permit such a comparison is being done in a group of monkeys, each weighing 12–14 kg, that compare with humans at 40–50 years of age.

C. Chang: The question we usually want to ask is, "Why the delayed radionecrosis?" I am surprised that, in the monkey's brain, 8,000 rads did not show more damage than 6,000 rads. We tried to study radiation exposure at a dose level of 1,000 rads in a single exposure, at which it would not produce any vascular change. Probably at this level no late vascular change would be demonstrated.

At a dose level of 4,000–5,000 rads, vessel changes are subtle. We counted each vessel 26 or 52 weeks after whole or partial brain irradiation in a cat. The response depends on whether the vessel is in the white or gray matter. A vessel in the white matter exposed to the same dose, within the same field, shows more changes in the endothelial wall than a vessel in the gray matter.

In Holland, Dr. Reinhardt has devised a radiobiologic method to determine the survival dose–response curve of endothelial cells. This ingenious method should supply information about the late-dose response, and eventually may provide the information Dr. Rubinstein is seeking.

We must establish the dose–response curve of endothelial cells of the blood vessels and try to determine the dose level that will not produce brain damage. On the other hand, we have to have some brain damage to achieve tumor control. I would like to know why no difference in brain damage was observed between 6,000 and 8,000 rads. Is the damage so extensive that the criteria used may not be stringent enough to dissociate those two groups?

Caveness: At 26 weeks, the lesions produced by 6,000 and by 8,000 rads are similar; after 8,000 rads, the lesions are a bit larger and extend deeper into the brain stem. However, between 26 and 52 weeks, there is a profound difference in the effects produced by these two dosages. With 6,000 rads, the necrotic lesions tend to mineralize; with 8,000, the lesions become larger and more confluent and go on to gross destruction at 52 weeks.

Rubinstein: This suggests that some kind of vicious circle is set up at a particular threshold. Drs. Zeman and Lampert have proposed that another element may be operating in these lesions, particularly at the late stage, i.e., an autoimmune phenomenon. Would you comment on that possibility?

Caveness: No, sir, I would not, but I would agree.

C. Wilson: Some years ago, Dr. Wilkins at Duke demonstrated a high frequency of neoplastic cells in the CSF of patients with gliomas after surgery, even the benign ones. Dr. Davis told us that 3 of his 20 patients with glioblastomas had clinically significant spinal cord seeding at the time of autopsy; presumably, all had been operated upon. What is the significance of the free-floating neoplastic cell in CSF, for example, as an indication for irradiation of the spinal cord, or in the patient failing to respond to therapy?

Rubinstein: There is an important biologic difference between the phenomenon of simple cellular seeding and that of metastasis formation, not only in the CNS but also elsewhere in the body. Some years ago, the late Dr. Harry Greene emphasized clearly that visceral organs that are apparently free of tumor may, on subsequent transplantation, demonstrate actual growth of metastases. Another factor is the time element. In an editorial in the *Archives of Neurology*, Dr. Alvord suggested that the absence of visceral metastases in gliomas may be determined largely by the insufficient time element related to the doubling factor in the growth of the primary tumor.

Some years ago, Dr. Thomas P. Morley of Toronto described tumor cells in the blood of patients operated on for glioblastomas; they were sought at or shortly after operation. Has this work been repeated or confirmed?

Wilson: As far as I know, this work was never confirmed. Most of these tumor cells floating in the blood have subsequently been dismissed as artifactual.

Rubinstein: Dr. Davis, would you care to comment on the significance of malignant cells in the CSF?

R. Davis: One paper in the German literature reports a 20% incidence of extensive spinal cord seeding. In most reports the highest incidence was 15%. The patient whose slides I showed was admitted with a diagnosis of encephalitis, and the diagnosis was supported by the presence of mononuclear cells in the CSF. He died without surgery being performed. So apparently seeding can occur early.

Mahaley: Were those seeded cases all treated with radiation or with chemotherapy?

Davis: The patient I just mentioned received no treatment. The other two patients had surgery, but I do not know if they had additional radiation therapy.

S. Bogoch: Dr. Vogel, one of your slides showed active proliferation of cells around a cystic area in a malignant glioma. You suggested that this proliferation might account for the palisading effect, an explanation that is extremely interesting from the neurochemical viewpoint. For many years, Allen and his associates, Kirsch, and others have drawn attention to the anaerobic capabilities of brain tumor cells. This goes back to Warburg's original observations on the ability of malignant cells to exist with less oxygen, even in the presence of cyanide. If this is typical, the palisading may reflect the active growth of malignant glial cells in a relatively anaerobic environment.

F. S. Vogel: Two possible explanations exist. It is generally assumed that the necrosis is ischemic. We are moving from an area sufficiently ischemic to cause tissue death to an area that is marginally oxygenated and presumably has the highest density of cells. What we call "palisading" describes a local increase in cell population rather than a specific histologic arrangement.

The other possibility you have suggested is that this is an area where anoxia has stimulated hyperplasia. Perhaps the breakdown of cells in the area of necrosis has provided substrate that nearby tumor cells used for proliferation. Either or both seem possible.

A. Freeman: It has been suggested that some of the bizarre syndromes that follow radiotherapy and chemotherapy may be due to a cytolytic virus rather than to direct toxicity. Does radiation therapy increase the brain's susceptibility to challenge by cytolytic virus?

Vogel: If one looks "across the board" at the response in different animals to irradiation and different forms of irradiation, one is struck by the similarity, with few exceptions, of the tissue response. If this is so, your theoretical virus must be a pathogen in the normal brain tissue of these various species. That is an unlikely possibility.

Freeman: I did not make myself clear. Assuming someone is irradiated and shortly thereafter is exposed to a herpes simplex virus, will this person's brain be more susceptible to damage by this virus than would a normal one?

Caveness: A herpes virus present after irradiation will express itself clinically. So far, we have not been successful in our attempts to demonstrate in the monkey a virus inactivated by radiation. We have found some suggestive compact tubuloreticular formations on electron microscopic examinations of these tissues. In one of the monkeys (the first model), foamy virus was cultured in the irradiated side; it was not cultured from the nonirradiated side in the same brain.

Rubinstein: All centers that use extensive anticancer treatment encounter an increased association of opportunistic infection, particularly affecting the nervous system. The question is whether irradiation to the brain favors the appearance of an opportunistic virus. It is logical to expect it; the brain is privileged from the point of view of its immunologic tolerance. If you reduce by irradiation what little tolerance it has, to what extent does this stimulate the emergence of an opportunistic virus?

R. Blasberg: You are not suggesting that the lesions Drs. Caveness and Mahaley have described after radiation or chemotherapy are of viral origin. They are not the type of lesion one sees in progressive multifocal leukoencephalopathy, for instance.

Rubinstein: I am talking about demonstrable infection by agents that you can recognize.

Blasberg: Those who are not neurologists or neuropathologists could leave with the idea that these lesions may be viral, and that is not the case. The radiation or chemotherapeutic injury that was shown certainly involves the capillaries. However, a significant element of demyelination occurs. Are we dealing with a primary demyelinative process or demyelination secondary to the vascular change?

Finally, one of the two long-term survivors died of a similar lesion in the involved hemisphere.

Caveness: Some indication exists that vascular impairment precedes demyelinization. Once overt breakdown begins, demyelinization proceeds rapidly, accompanied by a necrotic reaction to the demyelinization. This necrosis can erase some of the vascular changes but leaves telangiectasia around the periphery.

Vogel: A vascular lesion is generally accepted as a forerunner of demyelinization, and myelin is extremely sensitive to ischemia. This fact led Putnam to suggest, a number of years ago, that multiple sclerosis represented an ischemic insult.

The basic biologic principle is quite correct; myelin is sensitive to ischemia. Most investigators, such as Zeman and Haymaker, believe that the vascular change is primary and the demyelinization is secondary, although there are exceptions to this. A few instances have been reported in which, after fast-neutron, high-energy irradiation to the brain, demyelinization appears that is out of proportion to the vascular change.

Blasberg: If it is a vascular and not a primary demyelinative change, why do we not see it in the cortex or see only limited lesions in the cortex?

Caveness: One does see these lesions in the cortex, first in the white matter but certainly in the cortex as well.

Vogel: The cortex has a better collateral circulation, so that if one vessel is occluded, the vascular supply is not compromised as severely as in the white matter. One long, penetrating vessel in the white matter can influence a great deal of tissue.

Rubinstein: One reason for this difference may be anatomical: in one primary demyelinating disease, acute hemorrhagic leukoencephalitis, the vascular lesions mimic radiation change. However, the primary targets are the veins and the venules, and those that are most severely affected in the brain are in the white matter.

Davis: Three patients have been reported to have died after fulminant courses beginning about 6 weeks after the end of radiation therapy; all had extensive demyelination in the area of radiation. In none was the radiation directed toward a primary CNS neoplasm; all had had brain stem lesions. Many have asked whether multiple sclerosis is triggered by radiation, or whether it is a virus disease induced by radiation. I did electron microscopy on a case some years ago and found no virus particles. Otherwise, it looked like multiple sclerosis.

Wilson: Do we have evidence to suggest some selective injury to oligodendroglia, and that it is not possible to repair or maintain the myelin?

Dr. Davis said that astrocytomas of the cerebellum run a benign course even after they are incompletely removed, and he suggested that the patient's life expectancy is little affected by the presence of the tumor. Indefinite survival after incomplete removal of an astrocytoma could have two explanations: the operation done to verify the diagnosis evoked some immune response that changed the character of the tumor; or this tumor has a limited growth potential, and the patient can contain it (this is the true definition of a benign tumor). This way of looking at these tumors helps us understand optic nerve gliomas and cerebellar astrocytoma. Would you develop this concept of the truly benign tumor?

Rubinstein: First, we have the question, "Are oligodendroglial cells the target in radiation?"

Caveness: Dr. Zeman believes that both the endothelial cells and the oligodendroglia are the target cells; the oligodendroglia are most likely to be involved in younger patients and the endothelial cells in the older.

Rubinstein: We are dealing here with a biologic continuum. Gliomas, like other tumors in the body, can grow in a benign fashion or in a malignant fashion. You cannot make a rigid separation between the two. Nevertheless, benign growth exists as a biologic phenomenon. This may be described as a growth that does not metastasize, remains circumscribed, and that grows slowly. The growth fraction is small and, after a time, most of the cells go into the G_0-phase.

We are familiar with this in other forms of brain tumors. The choroid plexus papilloma is generally a benign tumor. Many ependymomas are benign; they are circumscribed and grow slowly. All pathologists are familiar with subependymomas, which are incidental growths found postmortem but which occasionally may grow more aggressively. The cerebellar astrocytomas, the pilocytic astrocytomas of juvenile type, and the optic nerve gliomas constitute a biologic entity, i.e., slowly growing benign tumors that may arrest.

The reverse can happen too. A patient known to have a slow-growing tumor may have only epilepsy for many years: then a sudden acceleration in growth occurs with an increase of intracranial pressure, and the tumor is now clearly malignant. A tumor is not the same as a cell maintained in the laboratory, with growth and stationary phases. It is a phenomenon that may exhibit exacerbation, arrest, and sometimes increased anaplasia.

Among the major mysteries in human neoplasia are the factors that determine slow growth or sudden anaplasia.

H. Gilbert: Has anyone ever seen a patient with a malignant posterior fossa tumor who had a recurrence outside the fossa? In other words, does the seeding represent the biologic viciousness of the tumor, or is it just a dissociated event?

Rubinstein: If I understand you correctly, you wish to know whether we have seen proven posterior fossa tumors that later metastasized?

Gilbert: Seed, without recurring locally.

Rubinstein: Yes, it does occur.

Gilbert: I have polled most of the people in Southern California and it seems to be a rare event in the Los Angeles area.

Rubinstein: I think all of us in pathology have encountered at postmortem malignant gliomas, particularly medulloblastomas, which have shown extensive metastases after 3–5 years without gross recurrence at the primary site.

O. Salazar: Dr. Davis mentioned the magic word "astrocytoma, grade 2" and it is the first time I heard it here today. The original Armed Forces Institute of Pathology fascicle of Kernohan and Sayre discussed 5-year survival figures for each of these pathologic grades. Astrocytoma grade 2 did poorly, resembling grade 3 rather than grade 1. Dr. Kramer (at my institution) believes grade 2's resemble grade 3 more than grade 1. Dr. Vogel, on the other hand, discussed anaplastic astrocytomas and glioblastomas using three different terminologies. I am seeking agreement between these two terminologies: perhaps grade 1 is your astrocytoma, the anaplastic being grade 2 and 3, and the glioblastoma being grade 4?

My second question is for Dr. Caveness. I do not think anyone has given 8,000 rads to the human whole brain, but at the University of Rochester, we have given 5,000 rads and then boosted the primary lesion to target volume doses in the range of 7,000–8,000 rads. We have 29 patients; some have survived for 3 years and the longest survival is 4.5 years. These patients' lifespans are now coming to an end and we are getting their bodies for autopsies; there appears to be a correlation between the time that has elapsed when the radiation was given and the appearance of the damage described as "delayed late radiation reaction."

Caveness: We will be extremely interested in your autopsy findings. In the monkeys, the damage from 8,000 rads is time dependent.

Davis: I did not cite the statistics of Kernohan and Sayre because they include the cystic cerebellar astrocytoma and the juvenile type of pilocytic astrocytoma in their grade 1 lesions, and this biases the survival of the overall group significantly. I used only series that were confined to cerebral tumors because the great majority do not include those particular subtypes. However, in my work I do not use a grading system as Dr. Vogel does.

Vogel: Dr. Kernohan has expressed the same concept as Dr. Rubinstein, i.e., that we are dealing with a continuous spectrum of anaplasia. The classifications are quite arbitrary; Dr. Kernohan wished to use four divisions. I believe that once you see any of the features of a glioblastoma multiforme, that patient's clinical course is going to be short; therefore, one probably cannot subdivide the glioblastomas into a more or a less malignant variety. Also, subdivision is easier with three than with four categories.

Neuropathologists will separate certain entities, such as the juvenile type of astrocytoma, and attach a different prognosis to that diagnosis.

C. Leventhal: Dr. Rubinstein, when the gliosarcoma was first recognized, it was described as a collision tumor and regarded as an unusual phenomenon. Recently, I have had the impression that the more often you look for it, the more often you find it, and that this tumor is much more common than previously thought. If the circumstances under which it occurs can be defined, i.e., a sufficiently malignant glioblastoma with a sufficiently extensive contact between glioma and the meninges, is it a common or a rare event? A close study of the gliosarcoma might shed light on the mechanism of transfer of malignancy between two tissue types.

Rubinstein: May I call on Dr. Ransohoff to answer the question concerning the matter of incidence first, because he recently collaborated in an important clinicopathologic study of this phenomenon.

Ransohoff: I think Dr. Leventhal is correct when he says that the more often you look for gliosarcoma, the more often you become aware of it. Probably many primary glioblastomas occur, of which the unwary neuropathologist may say, "Well, there's a lot of connective tissue reaction around this tumor." Special study of that area will demonstrate that what he considered to be fibrous tissue reaction is really sarcoma.

This phenomenon does not depend simply on proximity to the arachnoid because it can occur in the depths of the white matter, where the sarcomatous change is in the fibrous tissue around the blood vessel. We have described a number of patients in whom the initial glioblastoma did not change, a repeat operation showed a mixed gliosarcoma, and autopsy revealed total sarcoma with no residual evidence of glioma. Therefore, the incidence is difficult to establish unless you search for it diligently. Dr. Morantz, who wrote the paper on our recent series of cases, is here.[1]

Rubinstein: Dr. Morantz, would you like to say something about this?

R. Morantz: A 20-year retrospective study of patients at the New York University hospitals revealed that the incidence of gliosarcoma in all gliomas is approximately 4%, and in glioblastomas alone, 8%.

Rubinstein: Dr. Morantz, I think you emphasized that the gliosarcoma is often localized in the temporal lobe.

Morantz: Yes, but I cannot explain this localization.

Rubinstein: I would like to come back to Dr. Leventhal's question, "Are these tumors more frequent than they were?" In this area, Dr. Feigin's work is seminal: he put these tumors on the map.

No evidence is given, as far as I can see, that an actual increase in the incidence of these tumors occurred, except that increased survival means a greater chance for this development to occur. The matter of tumor induction by one tissue type on another is fascinating; the reverse also occurs. It is much less common than in the glioblastomas with a sarcomatous change (the Feigin tumor) but occasionally malignant meningioma or a primary fibrosarcoma is found that infiltrates the glia and is surrounded by an unquestionably malignant gliomatous reaction. This phenomenon provides one of the most important pieces of evidence, from a morphologic point of view, against the somatic mutation theory in neoplasia. As Dr. Vogel mentioned, it has been most difficult to prove conclusively that this kind of tumor induction exists outside the nervous system. Neuropathologists are fortunate in one respect since they have delicate and specific histologic staining techniques at their disposal. They can differentiate fairly easily between a sarcoma and a glioma. It is much more difficult to do so in the rest of the body, but in the brain, the existence of true mixed tumors can be established with greater certainty, including the presumption of induction of one tumor type by another.

Leventhal: Intense desmoplastic reactions are not unusual in a variety of tumors. Dr. Ransohoff has commented on incorrect identification of a tumor in the early stages of discovery that might have sarcomatous elements in or around a carcinoma or lymphoma.

Rubinstein: Recently, we saw a cerebral neuroblastoma in a child. These tumors can show a marked desmoplastic reaction, like medulloblastomas. In the third recurrence of the growth, the desmoplasia was accompanied by a sarcomatous change. The tumor invaded the superior sagittal sinus and metastasized to the liver and vertebrae; in both areas, both tissue elements were found. This was a neuroblastoma that stimulated the production of a sarcoma.

Bogoch: In the brain of an animal that had an ependymoma glioblastoma growing under the skin of the abdomen, we demonstrated changes that are evidence of some sort of induction, i.e., the loss of long chains within 2–3 weeks, although there were no metastases in the brain. This is a distant effect.

With a variety of virus-induced sarcomas grown again subcutaneously or at other sites, Gray has shown evidence of malignant change in glycolipids of the kidney. These changes in the carbohydrate chains may be a much more common inductive effect than one would suspect from the current literature.

Chang: I would like to return to the important areas of irradiation, blood vessel changes, and host response. Haymaker and his colleagues, using the proton beam, irradiated 7 patients with glioblastoma, giving them 5,000 rads in 4 weeks. Of the 7, 4 were autopsied; the research team mapped out the area of irradiation carefully with India ink. They dissected the solid mass and studied it histologically and found no radiation damage to the normal tissue in the irradiated field outside the tumor. Biologically, 5,000 rads in 4 weeks is almost identical to 6,000 rads in 6 weeks. Maybe the proton beam differs from gamma radiation, but, according to their study, the proton beam is 1:1 with gamma radiation.

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[1] Morantz RA, Feigin I, Ransohoff J: Clinical and pathological study of 24 cases of gliosarcoma. J Neurosurg 45:398–408, 1976.

Caveness: The most interesting point to me is that these were protons rather than proton beams.

Chang: Concerning host response, one of my colleagues did a blood count on our patients receiving total-brain irradiation and found that all had selective lymphopenia.

M. Walker: Dr. Caveness performed the first animal toxicology study using radiotherapy, and he approached it much as one would approach drug toxicology. However, the ratio of drug toxicity in monkeys to that in man is about 3:1; that is, the monkey can tolerate about three times as much drug. Yet your dose–effect relationship curve shows about equitoxic dosimetry. How can this be? Has anybody studied other animals with single large doses as we have studied human beings?

Caveness: I do not know of any animal models currently being investigated with fractionated doses comparable to the therapeutic doses used in man.

Mahaley: Concerning the lymphopenia in patients with brain tumors, did Dr. Chang imply that whole-brain irradiation caused the lymphopenia? If blood counts are done on patients with brain tumors before either chemotherapy or radiotherapy, one finds that they have lymphopenia from the beginning. It is quite significant in the anaplastic gliomas and much less so in more benign brain tumors. If they are followed serially for a period after therapy has been started, a progressive and striking increase in the lymphopenia is noted, but only in those with anaplastic gliomas.

I do not know what role therapy plays in the progressive serial decrease in peripheral lymphocytes, but radiotherapy is not the sole cause of the lymphopenia because the patients have it before they are treated. However, it may play a role later in their disease. On the other hand, when radiotherapy is completed, and for months afterward, the lymphopenia becomes more severe as the patients are followed. We know little about what whole-head radiation does to patients in terms of their blood counts and their immune responses.

Chang: We observed that the lymphopenia disappeared in about 3 months after radiotherapy in all the patients.

A. Evans: Recently, I saw data from the acute leukemia trial UCAL–2 now being conducted in the United States. In that study, which compared different forms of prophylactic irradiation for meningeal leukemia, the researchers not only saw considerable lymphopenia in patients receiving radiation to the spine, but the marrow depression was almost equal in those who had irradiation of the head alone. These were children who, presumably, did not have any disease in the head and started their courses of irradiation with reasonable lymphocyte counts.

Mahaley: What were their counts before radiation was started?

Evans: About 3,000/mm^3.

Mahaley: It is possible that whole-head radiation does produce a change. A patient is under the cobalt beam long enough each day to have a large portion of the circulating lymphocytes exposed to the beam.

H. Abelson: Was there any evidence of immune dysfunction?

Mahaley: All we know is that lymphopenia is present before radiation and as one follows patients after radiation. In 40 patients we found that it declines progressively. They also have other anergies that I will not go into at this point.

B. Patrick: Dr. Vogel, in the mechanism of malignant transformation of tissues adjacent to glioblastomas, what role, if any, does the tumor angiogenesis factor play in this? Is this part of the continuum or is it something wholly unrelated?

Vogel: One sees proliferation of fibroblasts and of endothelial cells, and this may be brought about in a similar manner. When tumors seed into the subarachnoid space, and the blood vessels in the arachnoid above are examined, not only the vessels in the brain but also the vessels in the arachnoid show the capacity to proliferate endothelial cells.

Rubinstein: Incidentally, not only is this presumed tumor angiogenesis factor associated with gliomas within the CNS but it can be demonstrated occasionally in those that metastasize outside the brain. I have seen at least two examples (one in the lymph nodes, and one in the lung) that showed the same vigorous vascular endothelial proliferation in the stroma of a metastasizing malignant glioma. This suggests that the condition may be something fairly specific to the malignant glial cell in these instances.

Abelson: Has that been assayed for tumor angiogenesis factor?

Rubinstein: Dr. Patrick has raised the possibility of a tumor angiogenic factor that I think has been demonstrated recently in neural cells in culture. A report in *Science* about 6 months ago showed that this factor was produced by human astrocytoma cells, by C-6 rat glial cells, and by some neuroblastoma cell lines maintained in vitro.

Conventional Methods of Diagnosis Using Nuclear Scan and Recent Progress With Radionuclide Transaxial Tomography[1]

R. Edward Coleman[2] *and* **Malcolm D. Cooper**[3,4]

ABSTRACT—Radionuclide brain imaging, a simple, noninvasive test for evaluating patients with suspected cerebral pathology, has a high degree of accuracy when compared with more complex neuroradiologic procedures such as angiography and pneumoencephalography. The detection of brain tumors with the use of radionuclide scans is related to the tumor cell type and their location in the brain. Transmission computed cranial tomography (CCT) offers another simple procedure for evaluating cerebral pathology. Even though CCT gives more anatomic detail than the radionuclide study, the two procedures are often complementary. Radionuclide transaxial tomography appears promising as a means of evaluating cerebral metabolism and metabolic changes associated with cerebral pathology.—Natl Cancer Inst Monogr 46: 97–108, 1977.

The brain scan and electroencephalogram (EEG) used in conjunction with cerebral angiography and air contrast studies have become standard diagnostic methods in the management of patients with suspected cerebral disease. In this paper, we will discuss the use of these techniques for detection of brain tumor, with emphasis on the role of the radionuclide brain scan. Transmission transaxial tomography now appears to have an extremely useful diagnostic role in cerebral disease. In light of this, recent progress with radionuclide emission tomography will be demonstrated and its potential diagnostic implications discussed.

THE RADIONUCLIDE BRAIN SCAN

The standard five-view brain scan is obtained with either an Anger camera or rectilinear scanner (fig. 1A). The current radiopharmaceutical of choice, Tc-99m pertechnetate (15–20 mCi), has been available since 1964 (*1*). The patient is prepared for the study with 400 mg potassium perchlorate given orally. Atropine also may be used to reduce oropharyngeal activity in the vertex view. The five scan views are obtained approximately 2 hours after injection of the radiopharmaceutical. If the radioactivity is administered as a bolus injection, rapid scintiphotos taken at 1- to 2-second intervals provide sequential images of the radiopharmaceutical as it is distributed through the cerebral hemispheres (fig. 1B). This cerebral isotope angiogram may provide valuable differential diagnostic information. The entire radionuclide procedure does not stress the patient because the flow study is usually completed within 30 seconds of injection, and each scan view can be obtained in 5–8 minutes. Rarely, restless patients may require sedation, and with children or uncooperative patients, the head is supported during the study. The pertechnetate brain scan is nonspecific in that uptake of the radiopharmaceutical by cerebral lesions is a function of their vascularity, extracellular fluid volume, or degree of breakdown in the blood–brain barrier within and around the lesion. Figure 2 shows positive brain scans in four different types of cerebral lesions: an ischemic stroke, an abscess, an arteriovenous (AV) malformation, and a meningioma. Certain scan characteristics of these lesions may be helpful in differential diagnosis; e.g., the ischemic lesion is confined to the distribution in the cerebral artery, in this instance, the middle cerebral. The abscess, although in an atypical location, is beginning to show a ring-like configuration that may also be a feature of necrotic tumors. The AV malformation demonstrates an irregular configuration extending peripherally over the region of the parietal cortex. The large vessel draining the lesion is seen meandering to the base of the brain. The meningioma

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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[4] The authors thank Drs. J. E. Reed and J. P. Williams for their assistance in preparation of the case studies.

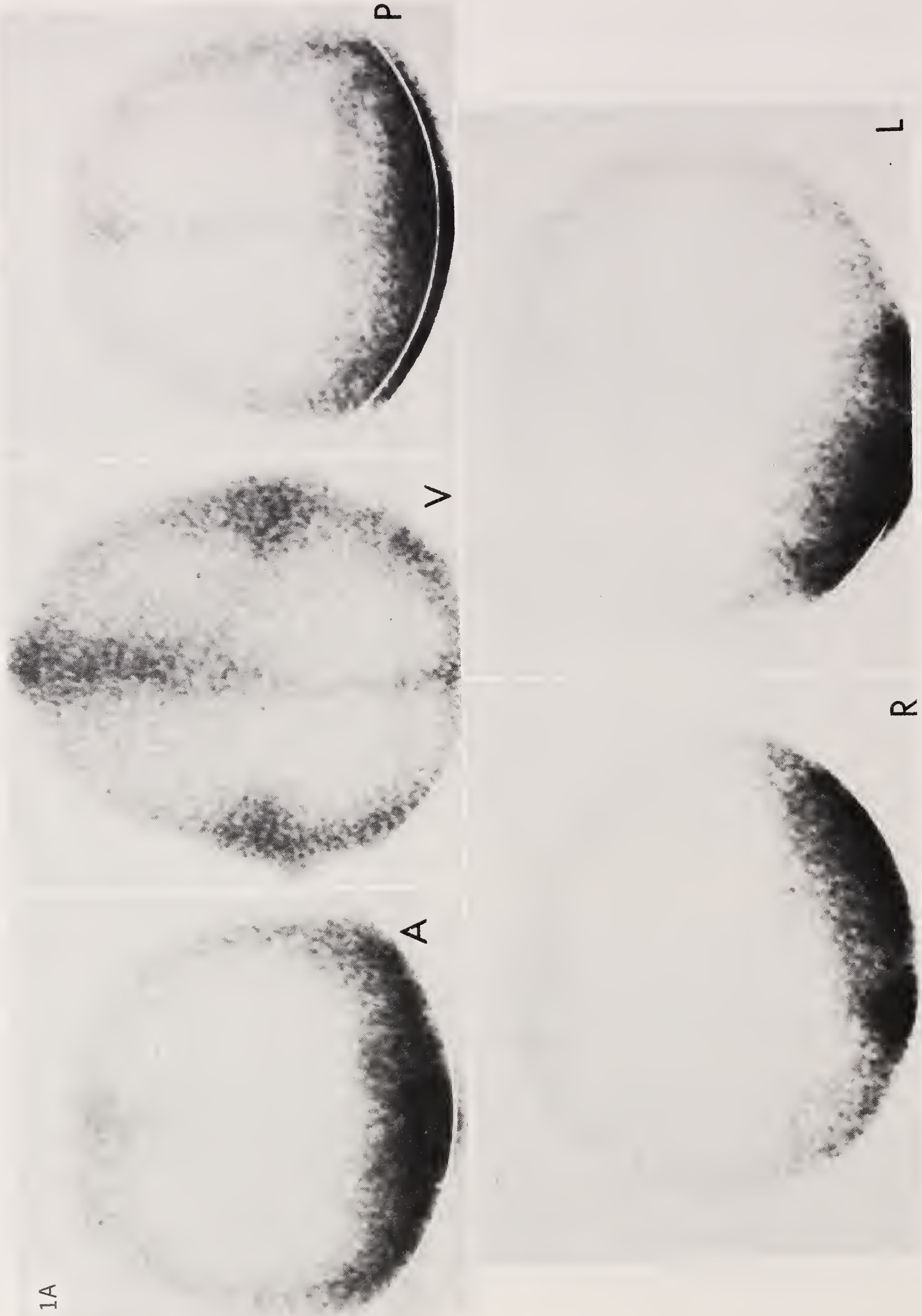

FIGURE 1A.—Normal brain scan (A = anterior; V = vertex; P = posterior; R = right lateral; L = left lateral). *See* text.

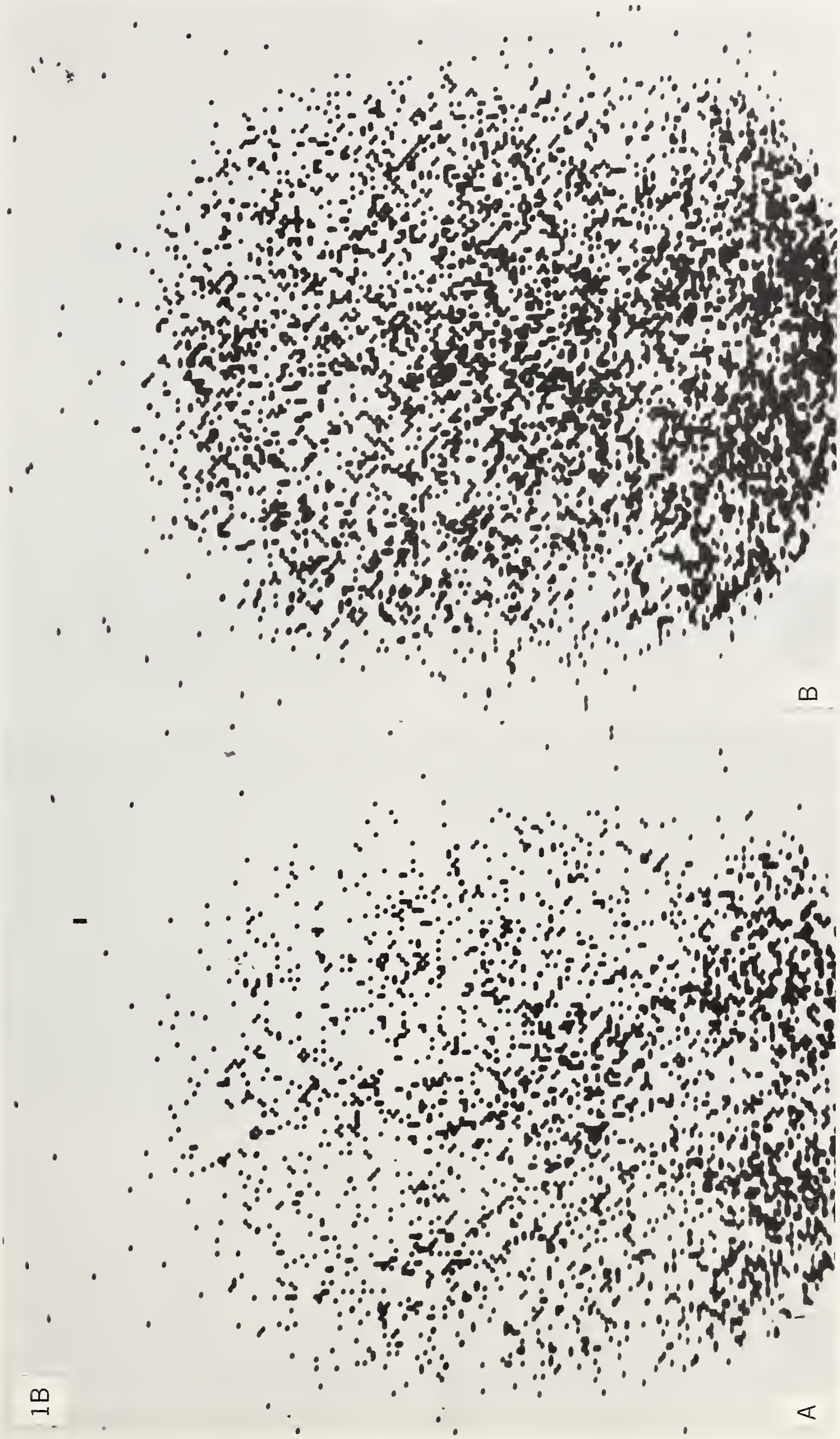

FIGURE 1B.—Normal brain scan, anterior. A = arterial phase; B = venous phase. *See* text.

is located peripherally, circumscribed and, in this instance, is located parasagittally against the falx in the anterior parietal region.

CLINICAL STUDIES

The following clinical studies illustrate the use of brain scans in patients with various cerebral tumors.

Case 1: meningiosarcoma.—A 50-year-old man presented with a 2- to 3-week history of weakness of left upper and lower extremities. Neurologic findings were localized to the left extremities, which manifested generalized weakness.

The brain scan demonstrated an *en-plaque* lesion, a finding frequently associated with subdural hematoma (fig. 3). However, the lesion visualized on the flow study suggested that the differential diagnosis should include a highly vascular tumor.

The angiogram demonstrated a large, vascular tumor in the parietal region and a marked shift of the anterior cerebral artery to the opposite side.

At operation, the removed specimen proved to be a meningiosarcoma.

Case 2: meningioma.—A 53-year-old woman presented with severe, persistent headaches that began approximately 4 months before admission. The only findings on physical examination were mild papilledema and some weakness of the left lateral rectus muscle.

An area of increased activity in the region of the sphenoid wing on the left was observed on the brain scan (fig. 4). This also was seen on the left lateral view.

On the lateral projection, the arteriogram demonstrated posterior displacement of the supracavernous portion of the anterior cerebral artery. On the frontal projection, there was upward bowing of the supracavernous portion of the anterior cerebral artery and also upward displacement of the middle cerebral artery on the same side. The preoperative diagnosis, sphenoid wing meningioma, was confirmed at operation.

Case 3: metastatic carcinoma.—A 56-year-old white man with proved carcinoma of the lung was referred for a brain scan because of decreased mental status.

On the scan, we noted several interesting features. The lesion in the occipital areas was symmetrical, crossed the midline, and was extremely large (fig. 5). This was an unusual appearance, and a symmetrical vascular lesion must be differentiated from symmetrical invasion by tumor.

On the arteriogram, a vertebral artery injection showed that the tumor was supplied predominantly from the posterior cerebral artery and that it was distributed symmetrically in both occipital lobes. The right carotid injection demonstrated that a portion of the tumor was supplied by the middle cerebral artery.

The tumor probably metastasized from the lung to the corpus callosum and grew symmetrically to involve the right and left occipital areas of the cerebral hemispheres. Arteriography demonstrated a dual blood supply.

Case 4: oligodendroglioma.—A 26-year-old man was referred for study with a history of seizures since childhood. Clinical examination was negative. Skull roentgenograms demonstrated calcification in the right parietal region.

The brain scan revealed a large mass lesion in the same area.

An arteriogram showed a large mass in the right parietal region and displacement of adjacent vessels (fig. 6). There was downward displacement of the sylvian point of the middle cerebral artery on the anterior view that was characteristic of a right parietal mass.

The differential diagnosis included meningioma, oligodendroglioma, or a slow-growing astrocytoma. Considering the patient's age, an oligodendroglioma was the most probable diagnosis and this was confirmed at operation.

THE NUCLEAR SCAN, ANGIOGRAM, AND AIR CONTRAST STUDIES

Since it is simple and noninvasive, the radionuclide brain scan is used with electroencephalography as a screening test in patients with suspected cerebral tumor. The brain scan has a high degree of diagnostic accuracy. When compared with more complex studies, such as pneumoencephalography and cerebral angiography, relative accuracy figures for the brain scan vary between 77 and 84% in a number of different studies (*2–7*).

Table 1 shows a comparison from 5 studies

TABLE 1.—*Accuracy of brain scan compared with other procedures in proved intracranial neoplasms*[a,b]

References	Brain scans	Arteriography	Pneumoencephalography-ventriculography
Afifi et al. (*3*)	52/62 (84)	33/36 (91)	19/20 (95)
Goodrich and Tutor (*4*)	99/118 (84)	70/85 (82)	21/25 (85)
Overton et al. (*5*)	84/100 (84)	64/76 (84	20/21 (95)
Witcofski et al. (*6*)	75/92 (81.5)	60/65 (92.3)	31/33 (93.9)
O'Mara and Mozley (*7*)	73/88 (83)	64/74 (86.5)	21/22 (95.4)

[a] Data was adapted from O'Mara and Mozley (*7*).

[b] *Numbers in parentheses* indicate percentages.

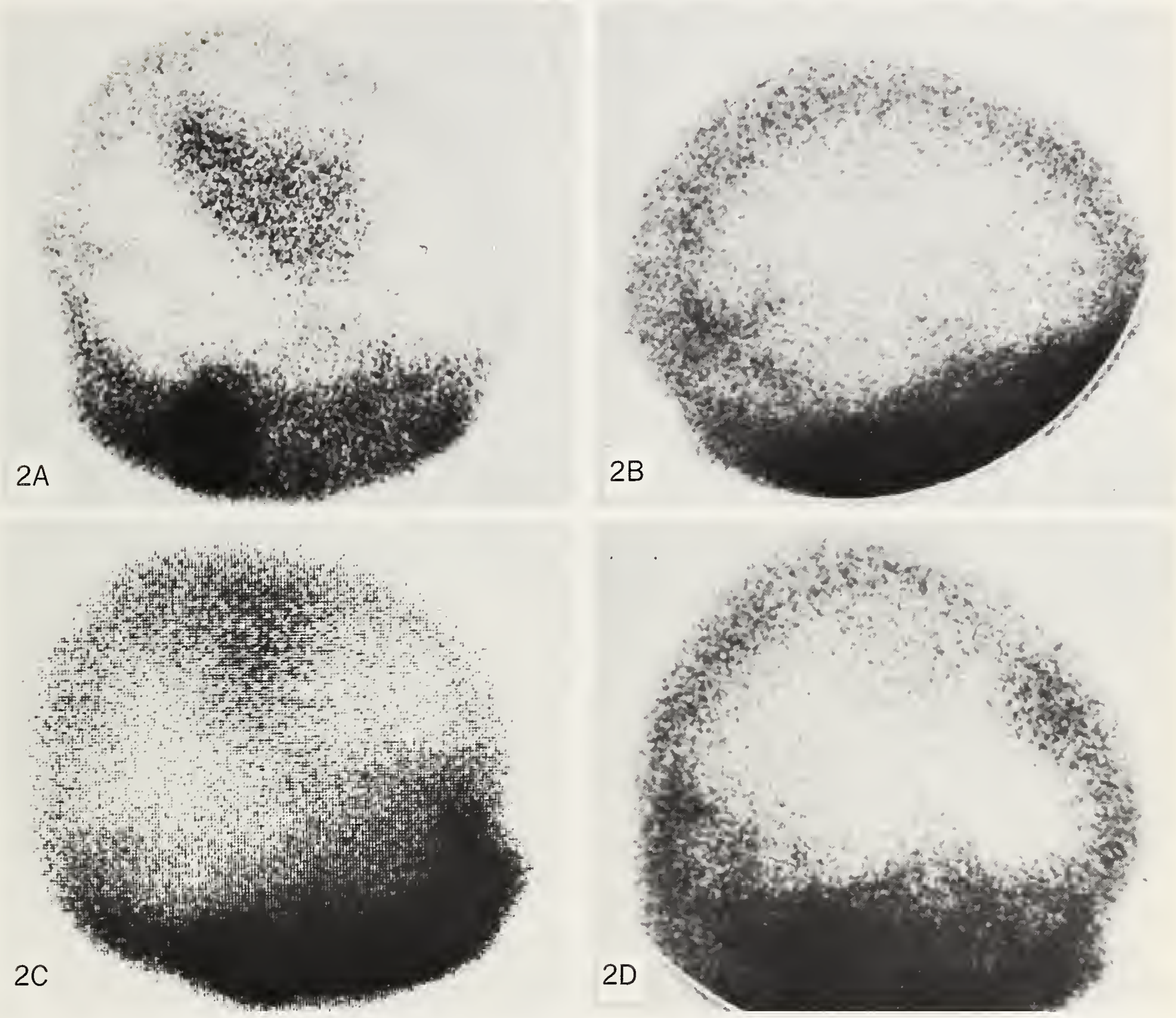

FIGURE 2.—Positive brain scan, various cerebral lesions: A = ischemic stroke; B = abscess; C = AV malformation; D = meningioma. *See* text.

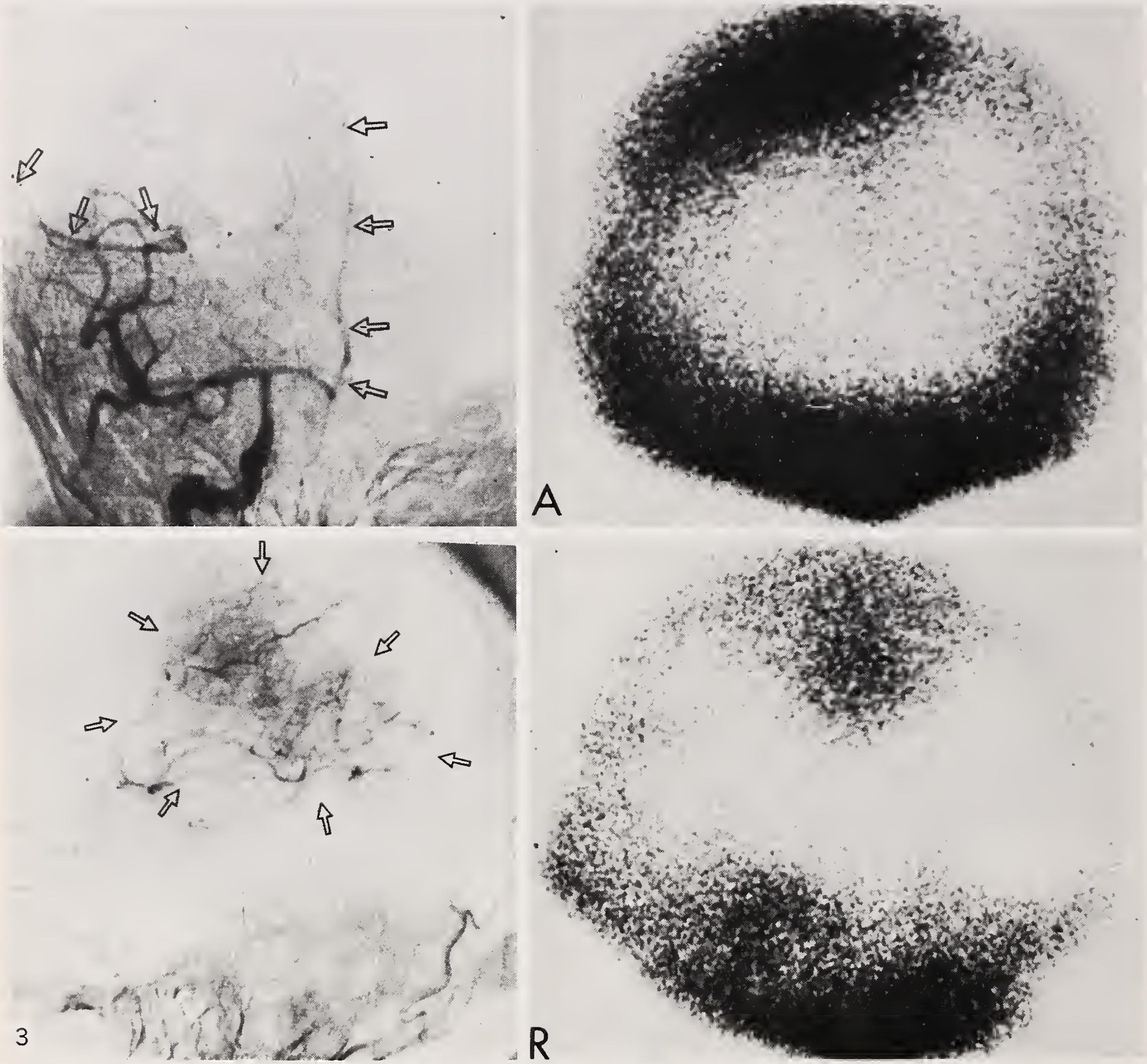

FIGURE 3.—Case 1, meningiosarcoma (A = anterior; R = right lateral). *See* text.

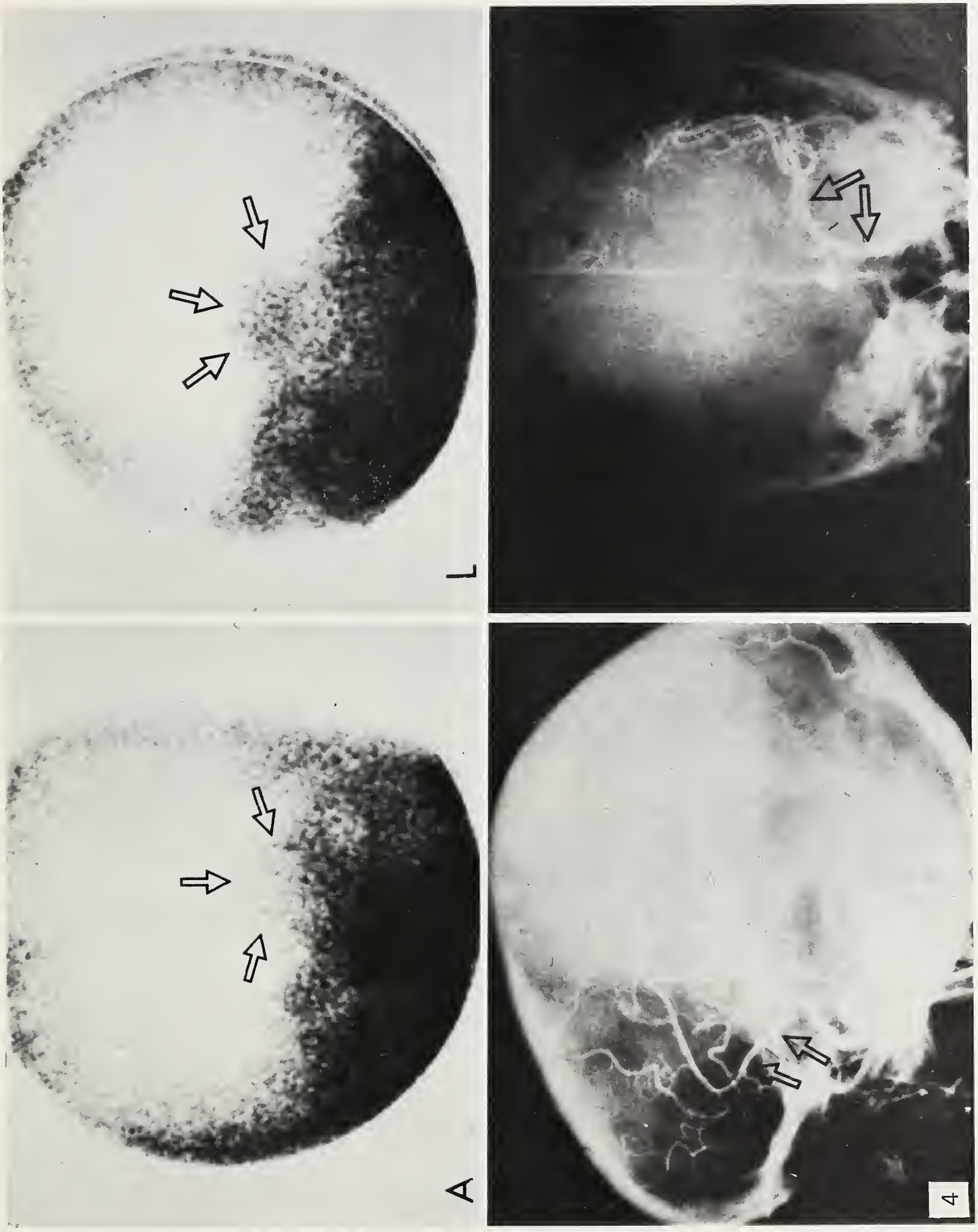

FIGURE 4.—Case 2, meningioma (A = anterior; L = left lateral). *See* text.

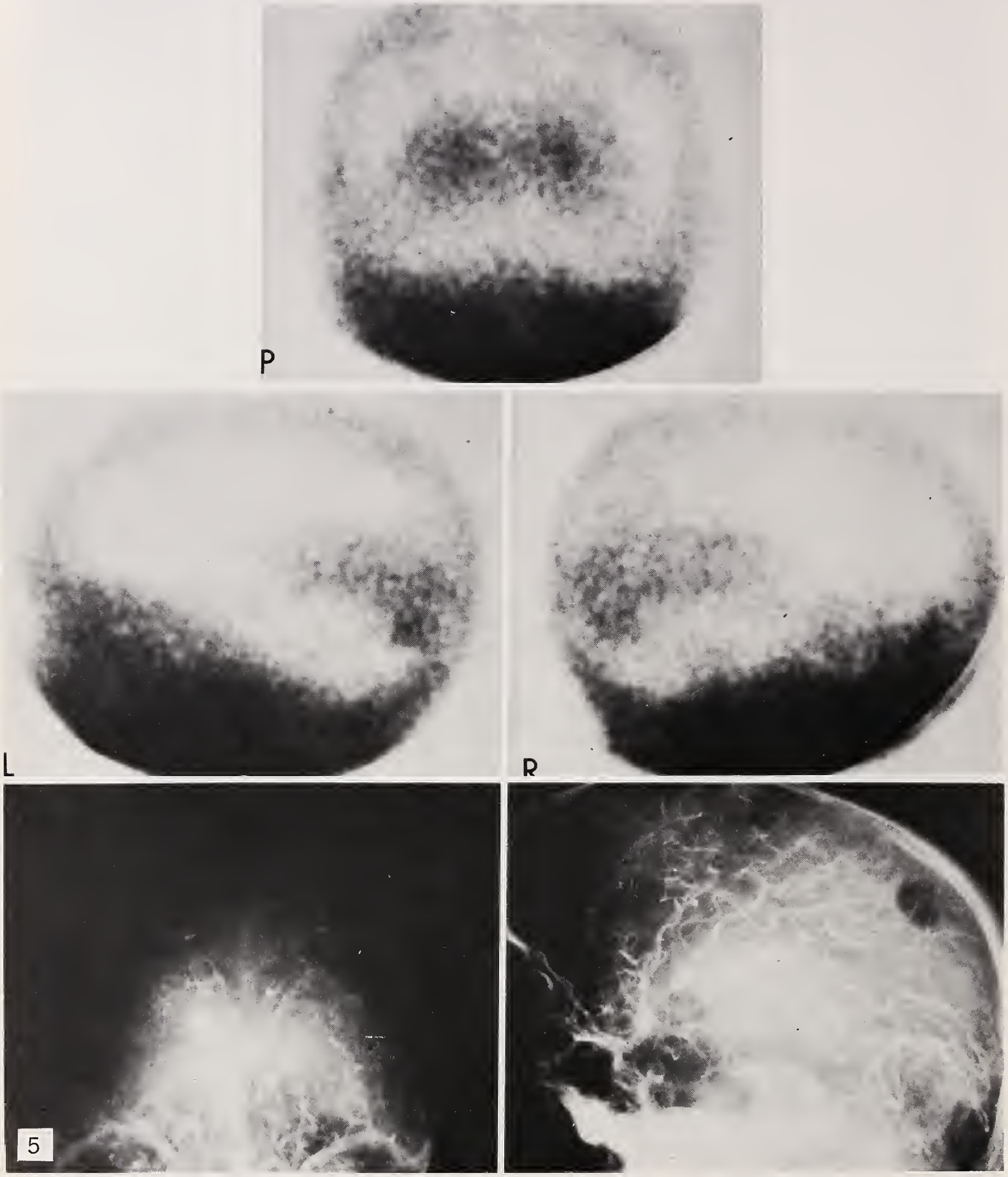

FIGURE 5.—Case 3, metastatic carcinoma (P = posterior; L = left lateral; R = right lateral). *See* text.

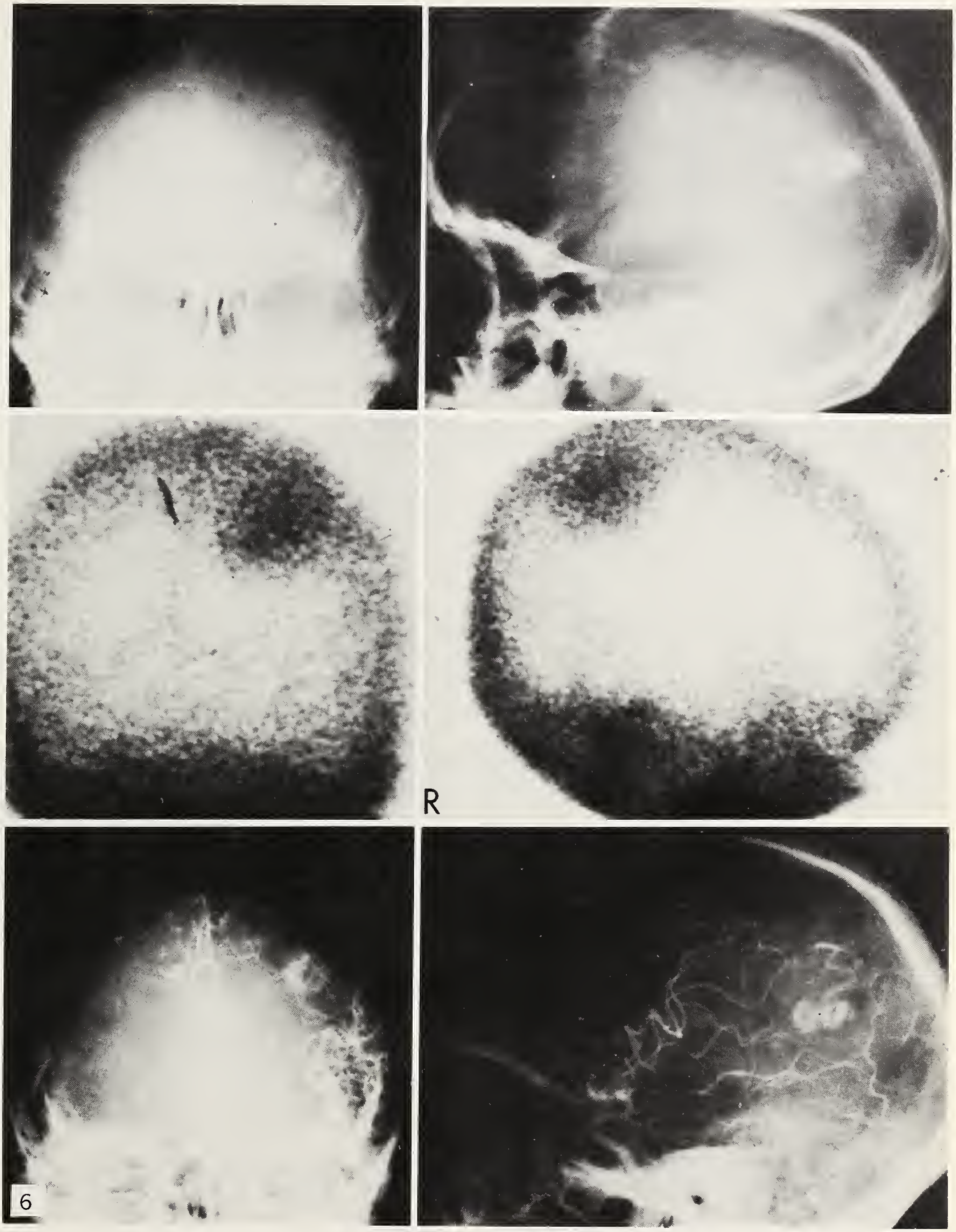

FIGURE 6.—Case 4, oligodendroglioma (R = right lateral). *See* text.

TABLE 2.—*Proved brain tumors studied with pertechnetate brain scans, cerebral angiography, EEG, and air studies*[a]

Diagnosis	No. of patients	Scan		Angiogram		EEG		Air	
		Positive/ total	Percent	Positive/ total	Percent	Positive/ total	Percent	Positive/ total	Percent
Astrocytoma	101	89/121	74	94/104	90	64/84	76	43/46	93
Meningioma	91	100/107	93	91/99	92	38/59	64	15/17	88
Metastatic	67	59/80	74	38/51	75	28/41	68	11/17	65
Pituitary adenoma	22	15/23	65	18/21	86	2/8	25	12/13	92
Acoustic neuroma	13	13/14	93	4/10	40	2/6	33	1/2	50
Craniopharyngioma	12	4/15	27	12/15	80	5/9	56	10/10	100
Ependymoma	11	8/12	67	6/10	60	4/8	50	7/8	88
Medulloblastoma	7	5/9	56	4/6	67	5/7	71	6/6	100
Optic glioma	4	2/4	50	1/2	50	—	—	1/1	100
Miscellaneous	4	3/4	75	1/4	25	2/4	50	1/1	100
Angioblastoma	3	1/3	33	2/3	67	—	—	—	—
Oligodendroglioma	2	3/3	100	3/3	100	2/2	100	—	—
Total	337	302/395	76.5	274/328	83.5	152/229	66.4	107/121	88.4

[a] Reproduced from (*2*) with permission of the publisher; *In* Noninvasive Brain Imaging: Computed Tomography and Radionuclides (De Blanc HJ, Sorenson JA, eds). New York: Society of Nuclear Medicine, 1975, pp 3–17.

TABLE 3.—*Accuracy of scanning, angiography, and air studies in relation to location*[a]

Location	Scan		Angiogram		Air	
	Positive/total	Percent	Positive/total	Percent	Positive/total	Percent
Frontal	68/88	77	53/65	82	14/20	70
Frontoparietal or parasagittal	35/36	97	26/28	93	5/5	100
Temporal	30/37	81	31/31	100	5/6	83
Parietal	68/75	91	56/63	89	15/16	94
Occipital	9/10	90	6/7	86	—	—
Thalamic, sellar, or parasellar	45/75	60	58/69	84	34/37	92
Sphenoid wing	14/14	100	14/14	100	—	—
Pons	3/12	25	7/9	78	5/6	83
Cerebellopontine angle	19/20	95	8/14	57	4/5	80
Medulla or cerebellum	26/49	53	18/34	53	31/34	91
Total	317/416	76	277/334	83	113/129	88

[a] *See* footnote, table 2.

that comprise a total of 460 patients with proved cerebral neoplasms. Table 2 shows the results from a recently published single review of 337 patients studied over a 10-year period between 1962 and 1972 (*2*). In this study, the relative diagnostic accuracy was 77% for the radionuclide scan, 84% for arteriography, 66% for electroencephalography, and 88% for air contrast studies. Table 3 from the same authors shows an interesting analysis of diagnostic accuracy of these 3 methods as a function of tumor location. Frontal lobe tumors were detected most frequently by angiograms. All 3 techniques were highly effective in parasagittal tumors. Angiograms were 100% successful in detecting temporal tumors, compared with rates of 81 and 83%, respectively, for scans and air studies. The detection rate in parietal tumors was similar in all three types of studies. No air studies were done in occipital tumors but in these lesions, scans and arteriograms were equally effective (90 and 86%, respectively). Thalamic, sellar, and parasellar tumors were detected more accurately by air studies, but both scans and arteriograms detected 100% of sphenoid wing meningiomas. Of the various anatomic regions, scanning was least effective in detecting pontine tumors (25%), compared with air studies (78%) and arteriography (80%). In contrast, scanning was more effective in detecting cerebellopontine angle tumors than either of the other methods. Air study demonstrated 91% of medullary and cerebellar tumors, compared with 53% in both scans and arteriograms.

In summarizing their experience, these authors (*2*) concluded that scanning was most effective in detecting meningiomas and acoustic neuromas. Angiograms were slightly more effective for other tumors, but were used less frequently because of the need for an intra-arterial injection. Air studies appeared to be the most accurate of the three diagnostic procedures, except for metastatic lesions, but these were used much less frequently because they were technically complex and were attended by much patient morbidity. The brain scan gave poor results in tumors of the brain stem and posterior fossa but were superior to the other studies in lesions in the cerebellopontine angle.

POSITRON EMISSION TRANSAXIAL TOMOGRAPHY (PETT III)

Recently, the new system PETT-III was developed at the Mallinckrodt Institute of Radiology for imaging positron emitting radiopharmaceuticals (*8*). This new imaging system determines the cross-sectional distribution of the radiopharmaceutical by an annihilation coincidence detection and a computerized reconstruction technique similar to that used for transmission computed tomography (*9*). This instrument can provide quantitative distribution studies (*10*). The system was developed to study the whole body, but the early clinical evaluation has involved mainly the heart and brain.

Several radiopharmaceuticals have been evaluated in patients with cerebral lesions (*11*). The cyclotron-produced radiopharmaceuticals that have been studied include $^{13}NH_3$ ($T\frac{1}{2}$ = 10 min), ^{11}CO ($T\frac{1}{2}$ = 20 min), and [^{11}C]glucose ($T\frac{1}{2}$ = 20 min). Generator-produced [^{68}Ga]EDTA ($T\frac{1}{2}$ = 68 min) has also been studied. Following the iv administration of $^{13}NH_3$, the distribution reflects cerebral perfusion (*12*). Blood pool and local cerebral blood volume can be obtained following the inhalation of ^{11}CO (*12*). Regional cerebral metabolism can be evaluated with [^{11}C]glucose (*11*). Abnormalities of the blood–brain barrier can be detected with [^{68}Ga]EDTA.

The radiopharmaceutical used for the study of the individual patient depends on the type of information needed; e.g., $^{13}NH_3$, is useful in differentiating those with a stroke from those with a tumor. All patients with strokes have had well-defined areas of decreased perfusion. The PETT-III study has been particularly valuable when cranial computed tomography (CCT) demonstrated an area of decreased density on the unenhanced image and the neuroradiologist cannot differentiate infarction from tumor. Although only 50 patients or so have been studied with the cyclotron-produced radiopharmaceuticals and PETT-III, the results are encouraging and this technique may make it possible to study in vivo metabolism.

DISCUSSION

The use of the radionuclide brain scan has increased dramatically over the past 10 years, largely because it is atraumatic and relatively simple to perform. Established as the screening test in patients with symptoms suggestive of brain disease, its diagnostic accuracy in detecting brain tumors compares favorably with that of more complex invasive procedures such as angiography and air contrast studies.

Currently, the role of the radionuclide brain scan is being reevaluated as an increasing number of centers in North America acquire CCT. This modality produces high-resolution anatomic studies of the brain that assure its position as a valuable new diagnostic tool.

Preliminary comparative studies show that CCT gives detection rates of 89% compared with 86% for radionuclide imaging in cerebral mass lesions that included both vascular and neoplastic types (*13*). Although CCT missed 11% of these lesions and radionuclide imaging did not detect 14%, when both procedures were used together, only 3% of lesions were missed.

In most centers, CCT appears to have reduced the need for air contrast studies, although reports of its effects on the frequency of angiography and radionuclide scanning vary from center to center. Furthermore, radionuclide cisternography appears to complement CCT when used to determine the functional status of cerebrospinal fluid pathways in the presence of ventricular dilatation. For the future, this complementary role deserves emphasis because CCT provides exquisite anatomic detail, whereas the radionuclide brain studies measure associated functional impairment. Also, the quantitative analysis of the metabolic pathways of various radiolabeled substrates by PETT-III promises to yield a great deal of useful pathophysiologic information.

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Some Aspects of the Computed Tomography of the Head[1]

Irvin I. Kricheff, Joseph P. Lin, *and* Norman Pay[2]

ABSTRACT—Computed tomography demonstrates intracranial neoplasms so clearly that nearly all early work in this field was limited to descriptions of the appearance of various lesions. Only recently have investigators directed their attention to the medical implications of the physical and pathophysiologic phenonmena that characterize this remarkable new technique. This paper will discuss recent work in these two areas and its clinical implications, and will examine the application of computed tomography to the evaluation of the postsurgical and postradiation patient.—Natl Cancer Inst Monogr 46: 109–117, 1977.

In the past, it was considered that the attenuation coefficients, a measure of radiation absorption, of most soft tissues were the same as water. This conclusion was reasonable because conventional radiographic techniques were unable to discriminate among the contrast levels of various body tissues, being limited to a discrimination difference of 2–4% (*1*). Thus radiation physicists showed little interest in obtaining further information regarding tissue absorption factors.

The introduction of computed transaxial tomography (CTT) has made available a high contrast, quantitative technique capable of detecting differences in attenuation coefficients with an accuracy greater than 0.5%. In the relative scale used with the EMI scanner, a 1% change in absorption coefficients is equivalent to 5 EMI units (*1*). With this new level of sensitivity, we urgently need further knowledge regarding the attenuation coefficients of various body tissues and the effect of radiation photon energies on these tissues.

Phelps et al. and other workers (*2–4*) have shown recently that linear attenuation coefficients of various body tissues are significantly affected by the photon energy of the radiation beam. The EMI scanner uses a 120-kilovolt peak (kVp) radiation beam, which is equivalent to energy of approximately 65 keV. Phelps et al. (*2*) demonstrated significant changes in linear attenuation coefficients with variations in photon energy. Radiation absorption seems to be affected by three tissue parameters: tissue density, atomic number, and electron density. Tissue density seems to have the least effect. X-Ray absorption occurs by either the photoelectric or compton effect. For lower energy X-ray photons, the photoelectric effect predominates and is influenced mostly by the atomic number of the absorption media. At higher energy levels, compton absorption predominates and is influenced by electron density. At energies of 100 keV and above, nearly all absorption appears to be due to compton effect. Physical density does play some role in compton interactions, which depend on electron volume density that is the product of the physical density (grams per cubic centimeter) and electron mass density (electrons per gram). Future CTT applications may use differential energy absorptions to produce "subtraction" images of greater clarity.

Phelps' work (*2*) has shown that the linear attenuation coefficient difference between the white and gray matter of the brain is greatest at the low energies and steadily diminishes with increasing photon energy (table 1). At photon energies greater than 100 keV, the difference in attenuation coefficients of gray and white matter is close to zero. In the energy ranges used for the EMI scanner (120 kVp), the difference in attenuation coefficients between gray and white matter is about 0.5–0.6% relative to water, corresponding to a difference of 2.5–3 EMI units. Thus CTT can differentiate between gray and white matter only under the best conditions and to do so, the entire depth of the tomographic slice, be it 13 or 8 mm, must be filled with brain tissue, for the CTT image display consists of a cross-sectional distribution of attenuation coefficients that represent the mean values of absorption throughout the entire thickness of any given slice. Differences in attenuation that may be produced by the presence of low-density cerebrospinal fluid (CSF) or high-density calcium will alter significantly the attenuation coefficient demonstrated by reconstructive tomography. When the linear attenuation coefficient of an object is being measured only partially and the object occupies the depth

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TABLE 1.—*Linear attenuation coefficients as function of photon energy for various human brain tumors*[a]

Energy, keV					Linear attenuation coefficients (cm^{-1})			
	Edematous brain (MJ)[b]		Edematous brain (JP)[b]		Glioblastoma (IR)[b]		Glioblastoma (NR)[b]	
	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
27.4	0.4309	0.4312	0.4294	0.4301	0.4478	0.4492	0.4417	0.4417
59.5	0.2075	0.2077	0.2074	0.2069	0.2106	0.2107	0.2128	0.2141
136.3	0.1560	0.1562	0.1556	0.1558	0.1570	0.1580		

[a] Data were adapted from (*2*) with permission of the publisher.

[b] Initials are those of the respective patients.

of a given slice, the measured attenuation coefficient will be the average of the attenuation coefficient of the object and other structures within the slice. Clinically, this phenomenon, which produces errors in attenuation coefficient measurements, has been referred to as "the partial volume" problem.

If partial volume creates difficulties, why not make slices that are narrow enough to include only the structure of interest? Why use a 13-, 8-, or 5-mm thickness if slices of smaller depth would give more accurate absorption information about small structures? We cannot produce smaller slices with present technology, because the thickness of the slice is reduced by collimating the X-ray beam that reduces the total number of photons that pass through the studied object and subsequently strike the detector. A decrease in the number of photons results in a relative increase in the noise of the image. Text-figures 1A, B demonstrate the marked reduction in signal-to-noise ratio that occurs on the EMI scanner in going from an 8-mm collimator (the narrowest in common use) to a 5-mm one. Perhaps future development of more powerful radiation sources, better detectors, and newer reconstructive techniques will permit us to examine thin slices.

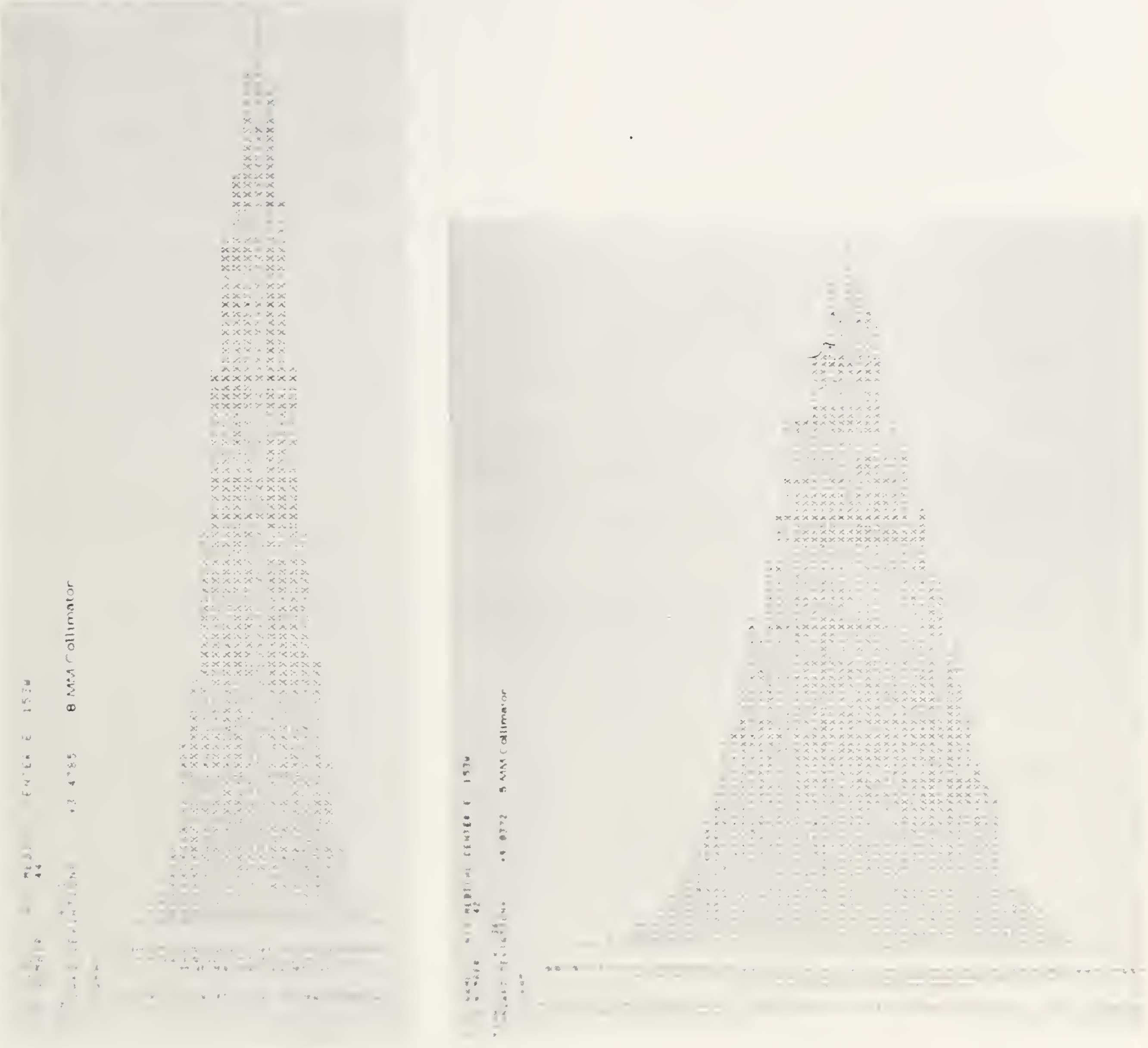
CONTRAST ENHANCEMENT

Early experience with CTT quickly revealed that numerous cerebral lesions were better visualized after the iv administration of iodinated contrast material (*5, 6*). The pharmaceuticals used for this purpose are the same as those used for excretory urography and angiography. Early studies showed that small volumes of contrast material, 30 cm^3 of 60% iodinated contrast, delivering between 9 and 10 g of iodine, sharply increased the absorption of meningiomas and some gliomas, but only occasionally did this technique improve the visualization of metastatic tumors. Subsequently, it was shown that larger doses of contrast material, i.e., 100 cm^3 of 60% solution delivering approximately 28 g iodine, greatly improved the visualization of neoplastic lesions (*7*).

Iodine solutions introduced into the systemic blood pool permit visualization of large cerebral vascular structures such as the circle of Willis and the straight sinus. Following such observations, many workers concluded that increased radiation absorption of lesions after the injection of contrast material was due to the increased vascular pool of cerebral neoplasms. However, tumors such as meningiomas, gliomas, and metastases, which have an extracerebral blood supply, receive their blood from dual sources; these tumors show a significantly greater contrast enhancement than do those deriving their supply solely from brain vasculature. These observations suggest that the blood–brain barrier inhibits contrast accumulation in tumors.

Recently, Gado et al. (*8, 9*) produced convincing evidence of an extravascular component of contrast enhancement. They determined tissue:blood ratio of contrast enhancement in 47 patients studied by CTT after first demonstrating a linear relationship between the attenuation values of different dilutions of contrast material. After a preliminary scan, a 6-cm^3 sample of blood was obtained, and 100 cm^3 of 60% methylglucamine iothalamate was injected iv for 4 minutes. A second scan was obtained at the end of the injection. Six-cubic centimeter samples of venous blood were obtained in heparinized syringes at the end of the first scan and at the beginning and end of the second scan. Brain contrast enhancement and blood samples were compared on the EMI printout in the same scanner. Tissue:blood ratios of tumors and normal intracranial structures were then calculated.

The increase in attenuation of blood due to the presence of iodine varied from 5.6 to 36.9 EMI units (*8*), with a mean value of 22.5 (±8.5). The mean value is that of an iodine content in the circulating blood of 173 (±65) mg/100 ml. Whereas structures such as the choroid plexus



TEXT-FIGURE 1A, B.—Signal-to-noise histograms produced from EMI scan data comparing: A) an 8-mm and B) a 5-mm collimator.

TABLE 2.—*Mean attenuation values and values of contrast enhancement of normal structures*[a]

Normal structures	No. of measurements[b]	Attenuation value before contrast injection[c]	Value of contrast enhancement[c]
White matter	62	16.2 (1.6)	0.6 (1.6)
Basal ganglia	14	17.7 (1.5)	0.6 (1.8)
Choroid plexus	9	27.5 (14.8)	6.3 (4.9)
Straight sinus	30	19.1 (3.0)	9.3 (5.3)

[a] Data are from (*8*); reprinted with permission of the publisher.
[b] In several cases, measurements of white matter were taken from more than one area of the same scan.
[c] Results are expressed as a mean (SD).

and the straight sinus, which are normally highly vascular, showed significant contrast enhancement after iv administration of contrast material, the values of the white matter and basal ganglia showed no significant changes (0.6 μ) (table 2)

The neoplastic lesions of 18 patients were from 1.9 to 24.2 μ (*8*). The tissue:blood ratios, which varied from 10 to 133%, were comparable irrespective of the histology of the lesion. If the tissue iodine within the field of scan was confined to the blood space, this ratio would have been equal to the blood volume per unit volume of tissues; however, it was much higher. In 12 of the 18 patients, the ratio was higher than 40% and in one glioblastoma and one meningioma, the ratios were 110 and 133%, respectively (table 3). Gado believes that these high tissue:blood ratios can be explained only by the presence of iodine outside the vascular space of the lesion. Furthermore in 10 other patients, values higher than 30% seem to be unrealistically high for cerebral blood volume because it is unlikely that the increased vascularity of these lesions could account for a 10- to 30-fold increase in the apparent cerebral blood volume.

TABLE 3.—*Contrast enhancement and tissue: blood ratio in neoplasms*[a]

Patient No.	Contrast enhancement		Tissue: blood ratio, %	Diagnosis
	Systemic blood	Lesion		
1	11.7	2.1	17.9	Glioblastoma
2	18.9	3.8	20.1	"
3	24.6	6.5	26.4	"
4	17.2	6.0	34.8	
5	28.0	16.5	59.0	"
6	14.6	10.1	69.1	"
7	16.8	18.6	110.0	"
8	18.5	1.9	10.3	Meningioma
9	10.2	3.4	33.3	"
10	17.4	8.2	47.1	"
11	18.2	24.2	133.0	"
12	17.3	10.3	59.5	"
13	40.6	8.0	19.6	Metastasis
14	30.2	7.2	23.8	"
15	15.4	6.6	42.9	"
16	16.4	6.8	41.5	"
17	19.1	8.0	41.9	"
18	17.5	10.5	60.0	Pituitary adenoma

[a] Data are from (*8*); reprinted with permission of the publisher.

Measurements of the ^{51}Cr-red blood cell volume were made in 2 tumors that showed a tissue:blood ratio of enhancement of 60.0 and 26.4% (*9*). The blood volume in these tumors was 10.1 and 7.8%, respectively; i.e., they were highly vascular lesions with values two to three times greater than normal. However, the contrast enhancement as measured by increased attenuation on the scanner was three to six times greater than the measured blood volume. This further supports Gado's observation that the values for tumor enhancement do not conform to blood volume. We can conclude that the iodine responsible for this enhancement is not confined to the blood space.

These data indicate that similar mechanisms underlie the abnormal accumulations seen with the radionuclide brain scan and the CTT contrast enhancement scan. If future work supports these observations, the complementary roles of the radionuclide brain scan and CTT may be narrower than had been anticipated.

Norman and Newton (*10*), who also studied the relationship between contrast injection and tumor-image enhancement, noted that there was no one-to-one relationship between the time of maximum enhancement and maximum blood levels; maximum enhancement occurred 20–40 minutes later except in vascular tumors. Performing sequential scans at 30- to 60-minute intervals, they demonstrated that the "necrotic center" frequently seen in malignant tumors may become enhanced on scans performed an hour or more after contrast injections. This suggests that these centers may not always represent necrosis, but may be a "second tumor compartment" into which contrast material passes at a slower rate.

Norman and Newton (*10*) also have evaluated drip infusion of contrast material versus bolus injection and reported that neither technique conferred any enhancement advantage. However, many radiologists believe that infusion produces less systemic symptoms than the more rapid bolus injection and hence prefer the former.

CLINICAL OBSERVATIONS

Surgical Evaluation

Postoperative neurosurgical patients may be divided into 2 groups in regard to their need for a CTT scan: 1) those with immediate postoperative complications, and 2) those to be evaluated concerning adequacy of surgical resection, recurrence of tumor, and/or ventricular size (*11*).

Patients whose condition deteriorates in the immediate postoperative period generally do not need to be studied by the contrast enhancement technique. CTT is performed to demonstrate an acute intracranial space-occupying lesion, and the important differential diagnosis is that between hematoma and edema. This differentiation was difficult by angiography but is made easily by CTT. On CTT, an acute intracerebral hematoma appears as a dense lesion, with an absorption coefficient from 20 to 45 μ, whereas cerebral edema presents as a lucent lesion with an absorption coefficient from 7 to 15 μU (fig. 1).

Patients evaluated for adequacy of surgical resection of intracranial neoplasia or recurrence of neoplasia must be studied after the iv infusion of contrast material, because it is impossible to differentiate consistently the lucencies produced by a surgical procedure from a recurrent lesion (fig. 2).

Radiotherapy Evaluation

The patient studied after radiation therapy should be examined routinely and with contrast enhancement to detect any changes in the cerebral neoplasm (*12*).

The CTT depicts tumor size and edema and their effect on surrounding intracranial structures. At times, radiation therapy produces changes in the intensity of contrast enhancement, with or without changes in tumor size. Occasionally, it may be difficult to correlate CTT with patient clinical status. Usually, the CTT relates well to clinical status (figs. 3A, B); however, sometimes little correlation is observed.

CTT is most helpful in detecting radiation edema and necrosis and in differentiating these from recurrent tumor. A high degree of suspicion is warranted if the scan picture of an area covered by the treatment field shows mottled lucency (CTT absorption coefficient of approximately 8–11) with extension to adjacent areas (fig. 4). It may be difficult to differentiate radiation edema from necrosis because both may manifest lower absorption coefficients than normal brain tissue, but several points may be helpful. If symptoms arise immediately after initiating radiation therapy, the CTT changes are more compatible with radiation edema; if they arise 6 months to 1 year or several years after such therapy, the changes are more compatible with radiation necrosis. Mass effect on the CTT of the affected side, with a shift of the midline structures, indicates the predominance of radiation edema over necrosis. The subsequent CTT may show a less prominent mass effect with a reversal of the density of the edematous brain toward normal, but the necrotic focus may remain lucent and continue to have a lower absorption coefficient than that of normal brain tissue. This differentiation between radiation necrosis and edema is still presumptive, but we are testing these concepts in the laboratory.

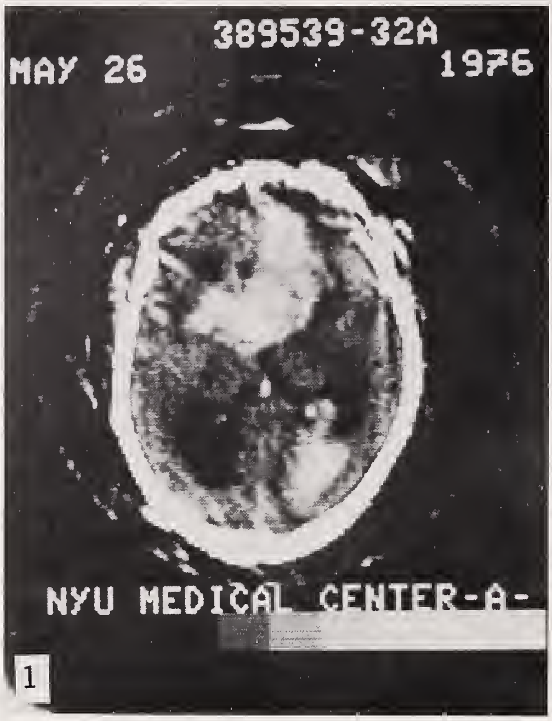
Contrast examination may not help to differentiate radiation edema from necrosis because both usually manifest a lack of uptake; however, areas of necrosis will occasionally enhance. This may be a confusing finding in the patient who has a recurrence of neurologic symptoms.

On CTT examination before and after radiation therapy, nearly half of all patients receiving doses of 3,000 to 6,000 rads to the whole brain show a symmetrical ventricular enlargement (figs. 5A, B). The ventricular enlargement appears 3–6 months after treatment and affects the lateral ventricles bilaterally. This phenomenon is as frequent in those who have had surgery as in those who have not. The ventricle contralateral to extensive surgical removal of brain tissue is usually as large as that on the surgical side.

Although we have no experimental evidence to support this finding, the work of earlier investigators suggests that radiation-induced cerebral atrophy is the most likely cause of postirradiation ventricular enlargement.

After pneumoencephalography, Wilson et al. (*13*) described atrophic changes in ventricles in patients who received irradiation to the cerebral hemispheres; the exact dosages were not mentioned. Robertson (*14*) described enlargement of the ventricles with reduction in the size of the cerebral hemisphere after radiation therapy despite evidence of residual tumor. In animal experiments, Haymaker (*15*) found that the threshold dose for radiation effects was 1,000 rads. Hicks (*16*) believes that during the course of fractionated X-ray therapy, atrophy changes appear when the brain has absorbed 3,000–4,000 rads. The endothelia of the blood vessels are affected first, then the neurons and glial cells are affected together. Demyelination occurs when the oligodendrocyte is affected. This depletion of cellular and myelin elements sets the stage for atrophic changes.

Increased CSF production secondary to irradiation (*17*) does not seem to be a convincing



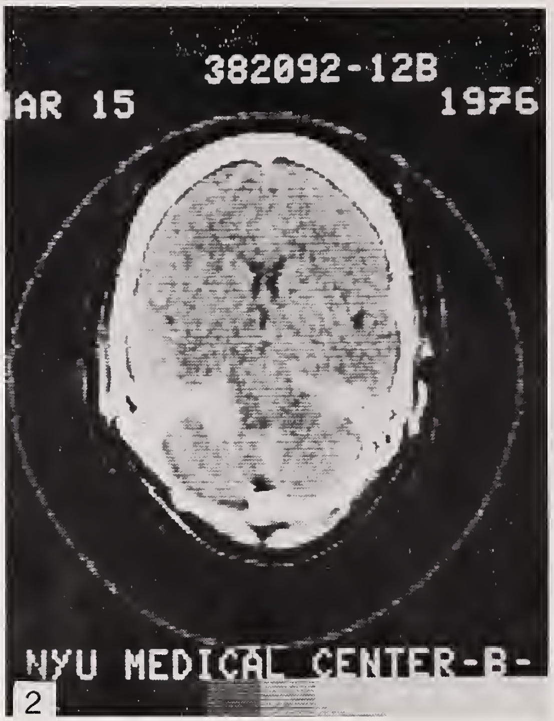


FIGURE 1.—Eight-hour postoperative nonenhancement CTT of a patient with bifrontal meningioma shows large right frontal hematoma (*white*) with surrounding cerebral edema (*black area* relative to normal brain). There is blood in both frontal horns and the right occipital horn.

FIGURE 2.—Two-year postoperative enhancement CTT of a patient with "total" removal of a left cerebellopontine angle meningioma shows recurrence of this meningioma with marked enhancement.

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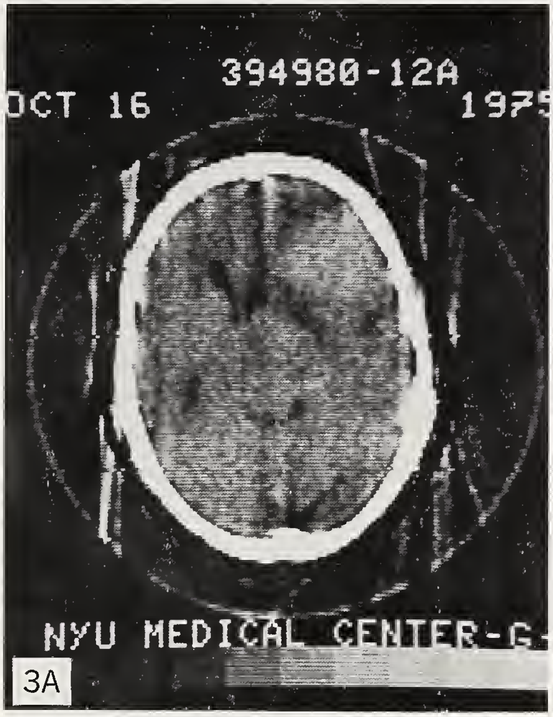
FIGURE 3A, B.—A) Pretreatment enhancement CTT shows right frontal astrocytoma that is markedly anaplastic and appears as a solid mass with surrounding edema. *Note* the marked displacement and distortion of the ventricular system. B) Enhancement CTT 1.5 mo after partial resection and radiation therapy: 4 MeV, tumor dose, 6,000 rads, 30 fractions for 6 wk through two opposed 12.0 × 17.5-cm fields. No evidence of tumor mass or enhancement. Ventricular system is in normal position. Lateral ventricles are slightly enlarged and there is some right frontal lucency. These findings are consistent with surgery. Patient was clinically well.

FIGURE 4.—Radiation edema and necrosis. CTT shows diffuse lucency throughout most of the left hemisphere corresponding to radiation treatment fields that spared 2.5 cm of the frontal and occipital poles. Patient received ^{60}Co therapy of 6,000 rads midline dose in 30 treatments over 40 days for a right parietal astrocytoma 1.5 years previously. Autopsy confirmed the CTT findings.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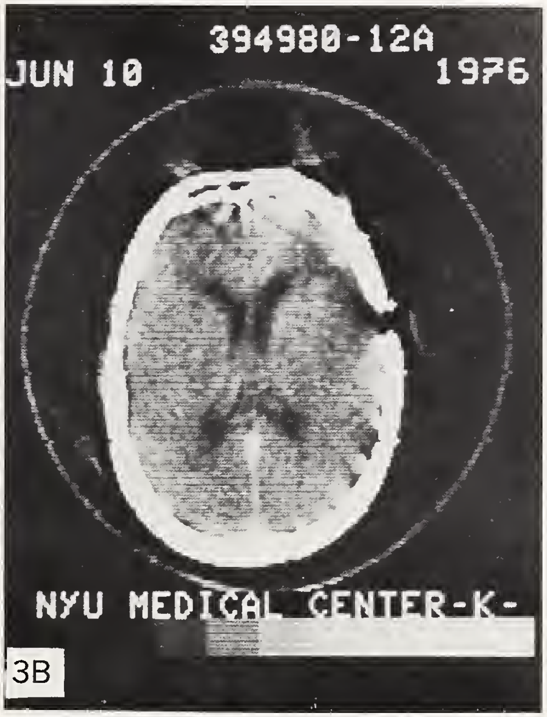
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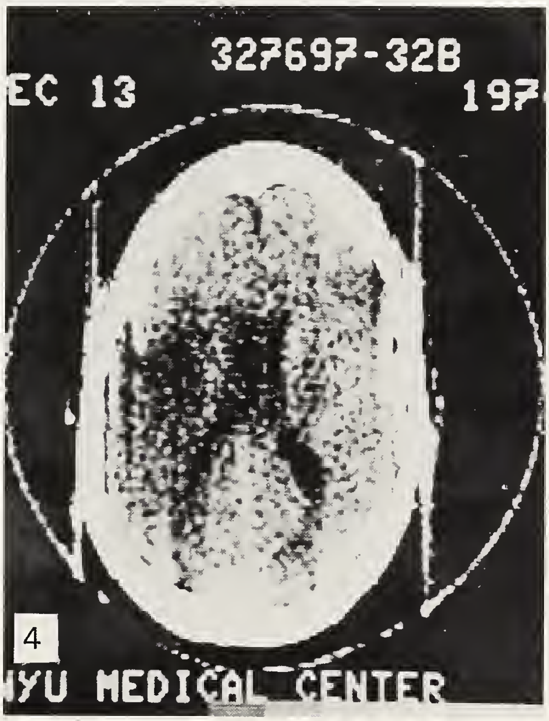
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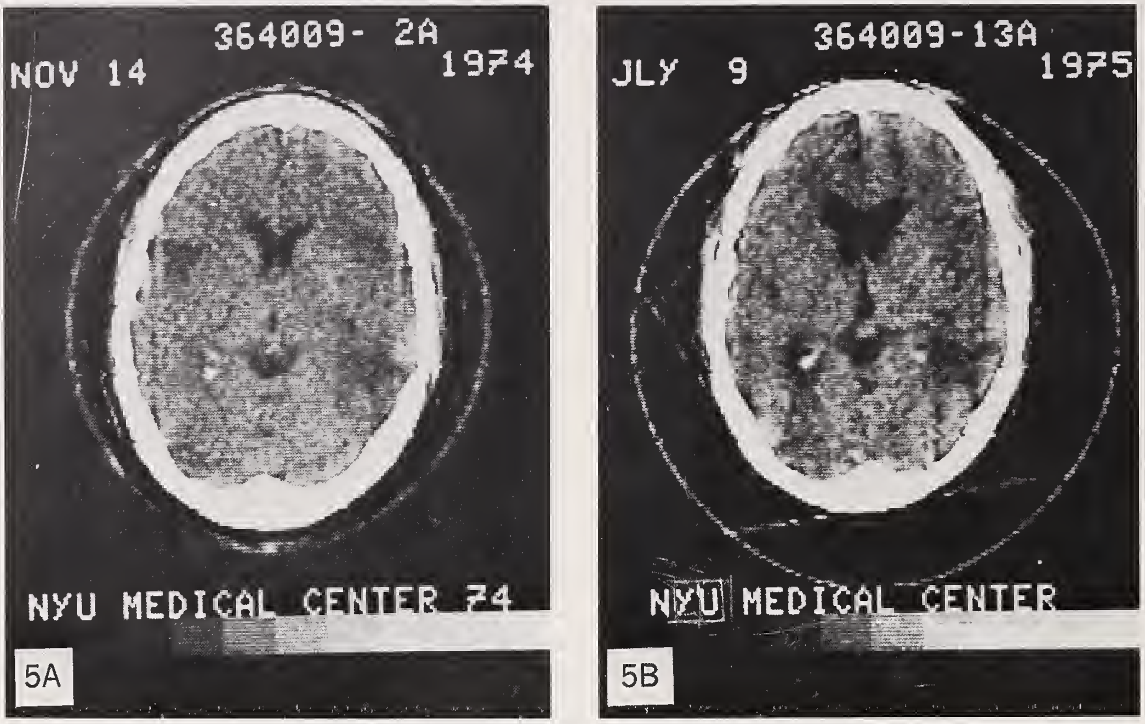

FIGURE 5A, B.—Postradiation ventricular enlargement. A) Pretreatment CTT. Left frontal and right temporoparietal metastases. Ventricles are normal size. B) Postirradiation enhancement CTT (4 mo). Lateral ventricles are significantly enlarged, and the left frontal lesion is no longer evident.

explanation of ventricular enlargement. This could be explained only if CSF production exceeded the absorptive capacity of the system, a situation in which the ventricles enlarge in response to increasing CSF pressure. Measurements of CSF pressure during and after radiation therapy have not demonstrated increased pressure. Redmond et al. (*18*) found no statistically significant pressure increase after 14 days of single doses of 1,000, 2,000, and 4,000 rads to the brains of dogs. Hakansson (*19*) also detected no significant increase of intracranial pressure that could be ascribed to irradiation.

The images produced by reconstructive tomography appear simple to interpret, and the diagnosis is often readily apparent. CTT is a complex tool, and its limitations have not yet been approached; it would be foolhardy to permit these simple images to lead us into simplistic thinking when dealing with these devices. We must not become mere technicians applying the developments of others but must work in close conjunction with the engineer and the mathematician to advance this exciting technology and to apply it for the benefit of our patients.

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Cerebrospinal Fluid Sterols in the Evaluation of Patients with Gliomas[1]

Joseph Ransohoff[2] *and* **Joseph Weiss**[3]

ABSTRACT—This study was an effort to obtain a marker indicative of the biologic activity of human gliomas. Sterol levels in the cerebrospinal fluid (CSF) were evaluated. Studies were conducted longitudinally during the course of tumor therapy. The evidence indicated that desmosterol levels in the CSF may reflect the growth potential of these neoplasms.—Natl Cancer Inst Monogr 46: 119–124, 1977.

Paoletti et al. (*1*) proposed a biochemical test for the diagnosis of brain tumors based on the observation that desmosterol (24-dehydrocholesterol) appears in the cerebrospinal fluid (CSF) of many patients with brain tumors after the administration of triparanol,[4] a drug that blocks the conversion of desmosterol to cholesterol. Desmosterol, the immediate precursor of cholesterol in the pathway of sterol synthesis in nervous tissue (*2*), is a major constituent of the developing brain when cholesterol biosynthesis is most rapid (*3*). Normal, mature human brain contains minute amounts of desmosterol (*4*), but it is present in increased quantities in various human and experimental tumors of the nervous system (*3, 5*). Total sterol synthesis is increased in neoplastic nervous tissue compared with normal tissue, and labeled precursor is incorporated into desmosterol to the greatest extent in malignant tumors, e.g., glioblastoma multiforme (*6, 7*). Since desmosterol can be found in the CSF of some patients with tumors, even without triparanol treatment, it appears to be synthesized within the tumor (*1*). However, it is not known whether plasma desmosterol also contributes to the increased desmosterol levels observed in the CSF of brain tumor patients after triparanol treatment.

We were prompted to apply this test to patients undergoing treatment for gliomas to determine whether the level of CSF desmosterol might indicate tumor activity. We studied a series of CSF samples obtained from individual patients at various stages of therapy: at the basal level, and after exposure to triparanol, surgery, radiation therapy, chemotherapy, or after a combination of treatments. The biochemical procedure described by Paoletti et al. (*1*) was simplified to adapt it to rapid, accurate performance in the clinical laboratory.

MATERIALS AND METHODS

Triparanol was administered orally for 5 days in a daily dose of 500 mg. CSF, 5–10 ml, was obtained by lumbar puncture after the patients had fasted overnight. Blood was drawn at the same time to determine the effect of triparanol administration on serum sterols.

The major modification of the procedure of Fumagalli and Paoletti (*8*) was to eliminate the lyophilization of the CSF sample and the thin-layer chromatographic purification of the sterols. The CSF was centrifuged for 20 minutes (2,500 rpm), and the supernatant was transferred to another sterile tube and frozen. Before analysis, the thawed and mixed CSF was again centrifuged to remove any sediment. Up to 10 ml CSF was added to glass-stoppered tubes to which 10 μg coprostanol had been added. An equal volume of absolute ethanol was mixed with the CSF, and then approximately 0.11 g of potassium hydroxide (in pellet form) for each milliliter of CSF was added with rapid mixing. The resultant mixture, equivalent to 1 N KOH in 50% ethanol, was heated for 90 minutes at 60° C.

The CSF hydrolysate was extracted four times with *n*-hexane and the combined nonsaponifiable extracts were evaporated under N_2 and vacuum. We redissolved the residue in a small amount of chloroform and dried it by passing the chloroform through a small column of anhydrous sodium sulfate; nitrogen was used to accelerate passage. The eluate and washings were collected in a microtube containing 5 μg of cholestane, which served as an internal standard in the gas chromatographic analysis. We prepared trimethylsilyl ether (TMS) derivatives of the sterols by mixing approximately 0.1 ml of the following mixture with the dried residue: pyridine, hexamethyldisilazane, and trimethylchlorsilane (9:3:1). After evaporation under N_2 and vacuum, the residue was dissolved in 50 μl carbon disulfide. Two microliters of the solution was analyzed with a gas chromatograph equipped with a hydrogen flame detector and a 4- or 6-ft, U-shaped glass column (2 mm, inner diameter) containing 1% neopertyl glycol succinate from Applied Science Lab., Inc., State College, Pa. (Hi–Eff–3BP) on 100–120 mesh Gas Chrom Q. The carrier gas was nitrogen and the column temperature was 200–225° C, depending on the length of the column. Good chromatographic separation between the cholesterol and desmosterol TMS peaks is essential. For the estimation of sterol concentrations, we prepared calibration curves under the same experimental conditions (*1*) by plotting the ratio of sterol TMS peak heights to cholestane (5 μg) peak height versus increasing concentrations of standard cholesterol or desmosterol. The ratio of the peak height of the TMS derivative of coprostanol (10 μg) and of cholestane (5 μg) was also determined. The addition of the second standard, coprostanol, before the saponification step corrects for any loss of CSF sterol during the procedure or for the incompleteness of formation of TMS derivatives.

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[4] Permission for the use of triparanol in this study was obtained from the Food and Drug Administration; it was supplied by the Wm. S. Merrell Co., Cincinnati, Ohio.

TABLE 1.—*CSF fluid sterols in patients with nonneoplastic neurologic disorders before and after triparanol administration*

Patient No.	Sex	Age, yr	Diagnosis	CSF[a]		
				Cholesterol, μg/ml		Desmosterol, μg/ml
				B	T	T
1	M	64	Organic mental syndrome	2.81	2.57	0.006
2	M	57	Head trauma (old) and seizures	4.14	3.52	0.014
3	M	64	Cervical cord compression: spondylosis	5.83	4.89	0.026
4	M	60	Cerebrovascular accident	4.67	3.97	0.019
5	M	63	Cerebrovascular accident	5.28	4.70	0.031
6	M	55	Seizure disorder	6.84	4.69	0.022
7	M	74	Parkinsonism	5.39	4.32	0.010

[a] B=basal sample before triparanol was given; T=after 5 days of triparanol administration; basal samples before triparanol were not detected for desmosterol.

The 0.5-ml serum samples were saponified with alcoholic KOH to which 5 ml distilled water were added, and the hydrolysate was extracted with 10 ml hexane. One milliliter of the hexane solution was dried and treated to form TMS derivatives of serum sterols for analysis by gas chromatography. The percentage of desmosterol and cholesterol present in the serum was determined by calculation of the areas of the respective peaks.

Blood in CSF results in abnormally high sterol levels. When the presence of blood was suspected, the following procedure with benzidine (*9*) was performed: A small amount of benzidine was dissolved in 1 ml glacial acetic acid, and 2 ml of 3% hydrogen peroxide was added. When this began to turn yellow, about 2 ml CSF (previously boiled and cooled to room temperature) was added. A green or blue color due to reaction of iron-containing derivatives of hemoglobin indicated the presence of blood.

RESULTS

The average cholesterol concentration in the CSF of control patients, i.e., those with nontumoral neurologic disorders, was 4.56 μg cholesterol/ml CSF (average of 9 pools taken from 47 patients). No desmosterol was detectable. A series of patients with nontumoral neurologic disorders was given triparanol for 5 days. Desmosterol was detected in all control patients after triparanol was given and averaged 0.018 g/ml CSF. One patient, chosen as a control, had a high increase in levels of CSF desmosterol, which suggested the presence of a tumor. Later tests confirmed this and indicated that the sterol test may be a useful diagnostic tool sometimes (patient #6, table 1).

The cholesterol levels of patients with gliomas generally were much higher than those of control patients, a finding that agrees with other observations that CSF cholesterol is increased in patients with intracranial tumors. The average cholesterol concentration in 40 patients with high-grade glioblastomas or gliomas at various stages of treatment was 15.60 μg cholesterol/ml CSF. Because most of the patients in our series had been operated on, it is difficult to determine the effect of surgery on the cholesterol levels; we have the impression that cholesterol levels generally were lower in patients who did not have surgery or had surgery some months before the CSF sample was taken.

A course of triparanol was given at least once to 34 patients undergoing treatment for gliomas. None of them responded to triparanol by showing an increased level of desmosterol. Some patients already had elevated levels of desmosterol in the basal samples of CSF and did not exhibit a further increase after triparanol. After comparing desmosterol levels in individual patients with gliomas before and after triparanol treatment (in relation to those levels of controls) and using a level of CSF desmosterol higher than 0.1 μg/ml to indicate the presence of tumor (as described by Paoletti et al.), we estimated that 60% of the patients in our series positively responded to desmosterol, whereas 20% showed a doubtful response. This response is lower that than obtained by Paoletti et al. in a series of patients with intracranial tumors who had received neither treatment nor surgery. At the present time, we cannot say whether either surgery or chemotherapy contributed to the reduced response to triparanol treatment observed in our patients (table 2).

Half the patients in our series were given 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) at the same time in their treatment. In those patients from whom two CSF samples were obtained (basal and posttriparanol), CSF desmosterol was usually increased after triparanol when BCNU was given concurrently with, or at a time shortly before, triparanol. In the 8 patients undergoing more prolonged chemotherapy with BCNU and from whom at least three CSF samples were obtained, the initial response to triparanol usually was limited, so that CSF desmosterol did not increase greatly after the first course of triparanol or following BCNU therapy and a subsequent course of triparanol. One patient, who had undergone surgery a year before his first treatment with triparanol, appeared to have a decrease in CSF desmosterol after a successful course of BCNU therapy.

DISCUSSION

An increase in CSF cholesterol is often an indication of an organic neurologic disorder, including neoplasms, (*10, 11*). Fumagalli and Paoletti (*8*) and we (this study) have documented an elevation of CSF cholesterol in patients with gliomas. In our series of patients with gliomas, no form of treatment showed a consistent effect on CSF cholesterol levels.

The level of CSF desmosterol, which is increased in patients with intracranial tumors after triparanol administration (*1, 8*), provides an additional indication of neoplastic growth in the nervous system. Our results confirm the validity of the "sterol test," in that desmosterol was elevated in 60% of our patients, all of whom had proved glioblastomas or gliomas of a high grade of malignancy. In a group of 27 patients with glioblastomas studied by Fumagalli and Paoletti (*8*), the test was positive in 82%; in 14 other patients, the incidence of positive response declined as the degree of malignancy of the gliomas decreased. The present series of patients differs from those studied by the Italian authors in that most had undergone surgery or had been treated by radiation, corticosteroids, chemotherapy, or a combination of these before or during the study. Although the biologic activity of the tumor, as affected by treatment, may be important in determining whether the test is positive, our data are not adequate to permit this analysis.

We assumed that the desmosterol that appears in the CSF after triparanol administration comes from increased synthesis within the tumor and is subsequently released into the CSF. Alterations of blood–brain and blood–CSF barriers produced by a tumor may facilitate passage of plasma sterols into the CSF. Even if changes in permeability did contribute to the appearance of cholesterol and desmosterol in the CSF, it would not diminish the potential usefulness of the sterol test. However, information concerning the importance of this factor would permit a more accurate application of the CSF sterol test, especially in the follow-up of patients. Also, we are interested in determining the effects of intracranial surgery on CSF sterol levels and are studying CSF sterol patterns in surgical patients with nonneoplastic conditions after triparanol administration.

The location of the tumor, especially in relation to the CSF pathways, appears to influence greatly the amount of cholesterol and desmosterol in the CSF (*8*). Human tumors, benign (acoustic neurinoma) and malignant (glioblastoma multiforme), accumulate desmosterol in situ after triparanol treatment (*12*). Patient 19 in this study demonstrates the importance of the location of the tumor: After triparanol treatment, this boy, who had a brain-stem glioma that seeded widely into the subarachnoid space, showed an extraordinarily high level of CSF cholesterol (40 μg/ml) and desmosterol (5.5 μg/ml). Whether certain types of tumors produce a high CSF desmosterol level because of their proximity to the CSF pathways or because they are associated with an increased uptake of sterols or triparanol from the peripheral circulation is not known.

In a study of some of the problems raised by the clinical study, we investigated the metabolism and transport of sterols in rats with tumors of the

TABLE 2.—*CSF sterols in patients with gliomas before and after triparanol administration*

				Treatment before CSF sample[a]			CSF[b]		
Patient No.	Sex	Age, yr	Diagnosis	Surgery date	Other date	Chemotherapy date	Date	Cholesterol, µg/ml	Desmosterol, µg/ml
15	M	62	Glioma	None			B: 6/70	6.27	ND
							T: 6/70	8.11	0.123
16	M	60	Glioblastoma	None			B: 3/70	3.97	ND
							T: 3/70	2.84	0.056
17	M	60	Inoperable glioma	None	R, S: 7/70		B: 7/70	11.31	0.015
							T: 7/70	12.58	0.180
18	M	55	Inoperable glioma	None	R: 7/68	Methotrexate: 1/69	B: 1/69	3.50	Trace
					S: 12/68	Mithramycin: conc	T: 1/69(7d)	3.06	0.227
19	M	10	Glioma, brainstem	None	R: 12/68	Methotrexate: 3,6,9/69	B: 4/70	39.66	0.040
					S: 12/68 3/69		T: 4/70	40.23	5.516
20	M	49	Recurrent glioma	1/67			B: 6/69	11.75	0.062
							T: 6/69	11.86	0.049
21	M	49	Recurrent glioblastoma	6/68	R		B: 6/69	5.68	ND
							T: 6/69	4.49	0.050
22	M	54	Recurrent glioblastoma	11/68	R	Mithramycin: 12/68	B: 5/69	3.81	ND
							T: 5/69	4.66	0.112
23	M	46	Glioma	6/69	R, S		T: 7/69	28.30	0.364
24	M	38	Glioma	2d: 7/69	S		T: 7/69	X17.16	0.556
25	M	44	Glioblastoma	2d: 12/69	S		B: 1/70	13.30	Trace
							T: 2/70	12.43	0.038
26	M	55	Glioblastoma	10/70	R, S		B: 10/70	10.28	0.016
							T: 11/70	7.83	0.120
27	M	58	Glioblastoma	10/70	R		B: 11/70	10.29	0.015
							T: 11/70	35.80	1.024
28	F	58	Glioma	6/70	R, S		B: 7/70	4.07	Trace
							T: 7/70	5.29	0.058
29	M	55	Glioblastoma	12/70	R		B: 12/70	12.72	ND
							T: 1/71	19.53	0.771
30	M	47	Glioblastoma	12/70	R		B: 12/70	12.67	0.020
							T: 1/71	20.84	0.331
31	M	59	Glioblastoma	11/69	S		B: 12/69	3.64	ND
							T: 12/69(4d)	8.68	0.005
32	M	63	Glioblastoma	5/70	R, S		B: 6/70	17.90	ND
							T: 6/70	14.76	0.307
33	M	64	Glioblastoma	9/70	R		B: 9/70	23.91	0.034
							T: 10/70	17.60	0.125
34	M	30	Glioblastoma	2d: 7/69	R, S	BCNU: conc	T: 8/69	55.32	0.220
35	F	60	Glioblastoma	7/70	S	BCNU: conc	T: 8/70	13.82	0.154
36	M	52	Glioma	2d: 4/69	S		B: 5/69	X31.54	0.227
						BCNU: 6/69	T: 6/69	24.50	0.195
37	F	62	Glioblastoma	12/70	S		B: 12/70	4.87	Trace
						BCNU: conc	T: 12/70	5.82	0.081
38	M	38	Inoperable glioblastoma	Biopsy: 2/79		BCNU: 2/70	B: 3/70	52.12	0.102
							T: 3/70	36.91	0.768
39	M	53	Recurrent glioblastoma	8/69	R	BCNU: 11/69	B: 1/70	14.36	ND
						BCNU: conc	T: 2/70	15.72	0.005
40	M	53	Inoperable glioblastoma	None	R: 12/69		B: 6/70	11.07	ND
					S: 1/12/70		T: 6/70	7.09	0.035
						BCNU: 6,8,10,12/70	T: 12/70	12.45	0.064
41	M	38	Recurrent glioma	6/68	R	BCNU: 6/69	B: 6/69	4.12	ND
							T: 6/69	6.68	0.101
						BCNU: 8/69	T: 2/70	4.50	0.013
42	M	48	Glioblastoma	2/70	R		B: 2/70	7.64	Trace
						BCNU: 3/70	T: 3/70	5.93	0.017
						BCNU: 5,7/70	T: 7/70	4.84	0.014
43	F	41	Glioma	2d: 1/70	S		B: 1/70	21.71	ND
							T: 2/70	34.49	0.020
						BCNU: 2/4/70; conc	T: 7/70	10.24	0.074

TABLE 2 (continued)

Patient No.	Sex	Age, yr	Diagnosis	Treatment before CSF sample[a]			CSF[b]		
				Surgery date	Other date	Chemotherapy date	Date	Cholesterol, μg/ml	Desmosterol, μg/ml
44	F	49	Glioblastoma	4/69	R, S		B: 5/69	10.35	ND
							T: 5/69	X31.47	0.327
						BCNU: 5/69; conc	T: 7/69	16.21	0.071
45	F	55	Glioblastoma	2/70	R, S		B: 3/70	5.58	ND
							T: 4/70	4.99	0.045
						BCNU: 4,6,8/70	T: 8/70	3.32	0.066
46	M	50	Glioblastoma	2/70	R		B: 3/70	8.32	ND
							T: 3/70	9.56	0.061
						BCNU: 3,5/70; conc	T: 8/70	5.69	0.043
47	M	47	Glioblastoma	6/69	R, S		B: 8/69	30.25	ND
							T: 8/69	30.86	0.568
						BCNU: 8/10/69; conc	T: 2/70	15.25	0.095
						BCNU: 5/70; conc	T: 8/70	6.53	0.230
48	M	48	Glioblastoma	2d: 9/69		BCNU: conc	B: 9/69	7.60	ND
							T: 9/69	13.08	0.035
							T: 2/70	4.30	0.039
							T: 4/70	8.00	0.058

[a] R=X-ray treatment postoperatively or as indicated; S=corticosteroid treatment postoperatively or as indicated; conc=chemotherapy administered concurrently with triparanol.

[b] B=basal sample before triparanol given; T=sample after 5 days of triparanol administration or as indicated; ND=not detected; X=blood contamination.

nervous system induced by nitrosourea derivatives. These tumors, which include neurinomas and gliomas, contain varying amounts of desmosterol (*5*). Preliminary results indicate that, compared with normal nerve or brain (*13*), malignant neurinomas of the intracranial portion of the trigeminal nerve induced in the offspring of rats treated with ethylnitrosourea may take up sterols from the plasma preferentially.

CONCLUSIONS

This work has been discontinued in our laboratories with Dr. Weiss' departure to laboratories of the Armed Forces Radiobiology Research Institute (Defense Nuclear Agency), but the search should continue for more specific tumor markers or brain-specific compounds that increase during malignant growth. Also, Christensen (*14*) reported that brain tumors contain increased quantities of glycosphingolipids. Probably the most useful of this group would be dihexose ceramide (cytolipin H) if it is shown to increase specifically in the CSF of patients with gliomas.

Since these substances have antigenic properties, immunologic techniques might be developed that would detect specific CSF changes. Certainly, the size of a glioma is not necessarily the true measure of its biologic activity. Sensitive measures of tumor activity would have great practical importance because indolent tumor may persist for many months following combination therapy before it escapes control and kills the patient.

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Chemotherapy of Human Nervous System Tumors: Influence on Cerebrospinal Fluid Sterols[1,2]

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ABSTRACT—The cerebrospinal fluid levels of cholesterol, desmosterol, and the ratio 100 D:C taken from a study of 59 patients are reported. The patients, who had tumors of the nervous system, underwent neurosurgery, radiotherapy, and chemotherapy with 1,3-bis(2-chloroethyl)-1-nitrosourea or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. The relationships among the levels of these three parameters and the clinical and neuroradiologic evolution are discussed.—Natl Cancer Inst Monogr 46: 125–126, 1977.

It has been reported previously that desmosterol, the last possible cholesterol precursor with an unsaturated side chain (*1*), accumulates selectively in the cerebrospinal fluid (CSF) of patients who bear nervous system tumors and who are treated with an inhibitor of the last steps of cholesterol biosynthesis (*2*). This specific accumulation of desmosterol in the CSF is correlated with the oncotypes (*3*) and has proved to be of diagnostic value for both the presence and recurrence of tumors of the nervous system (*4, 5*).

If it is assumed that the CSF sterol concentrations related to their biosynthesis in the tumor (*6*) can be correlated with the growth of the tumor itself, this biochemical parameter (CSF sterol test) can be used as a "spy" to monitor tumor evolution during chemotherapy.

We have used this test on patients with tumors of the nervous system who have been treated with derivatives of nitrosourea: 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). The antitumoral efficacy of these derivatives is already recognized (*6, 7*). Technical details for the measurement of CSF sterols have been reported (*8*).

Our case records to date include 59 patients who underwent neurosurgery (resection, partial removal, decompression, and/or ventriculo-atrial shunt, biopsy) and radiotherapy, followed by chemotherapy with BCNU or CCNU. The CSF sterols in these patients were determined. For 16 (11 treated with BCNU, 5 with CCNU), the CSF concentrations of cholesterol and desmosterol were followed by at least three successive determinations.

Earlier results have been reported and discussed elsewhere (*8*). Table 1 gives the characteristics of the new patients, and table 2 shows the trends of the CSF sterols in relation to the successive chemotherapy cycles. The results given in table 2 are for 5 patients whose neurologic condition was satisfactory during the various chemotherapy cycles.

The progress of the three parameters (CSF cholesterol, CSF desmosterol, 100 desmosterol: cholesterol) in 3 patients (12, 22A, and 38) is uniform and in agreement with the clinical evolution of the disease. In the other two, the concentrations of the two CSF sterols and their ratios are variable.

As was previously noted (*8*), we do not yet have sufficient information to indicate which of the three parameters most accurately indicates tumor evolution.

TABLE 1.—*Characteristics of the 5 patients*

Patient No.	Age, yr	Sex	Diagnosis	Surgery	Chemotherapy
10A	55	F	Frontal glioblastoma	Resection	CCNU
12	35	M	Temporal meningiosarcoma	Resection	BCNU
17	41	M	Cerebellar astrocytoma	Partial resection	BCNU
22A	47	F	2d grade parietal astrocytoma	Biopsy	CCNU
38	32	M	Cauda equina ependymoma	Resection	BCNU

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TABLE 2.—*Sterols in the CSF of 5 patients bearing tumors of the nervous system during chemotherapy*

Chemotherapy		Patient No.				
Cycles	Type, μg/ml	10A	12	17	22A	38
2d	Cholesterol	5.9	—	10.1	7.6	2.2
	Desmosterol	0.05	—	0.16	0.11	Not detectable
	100 D:C	0.8	—	1.6	1.4	—
3d	Cholesterol	—	—	—	2.4	—
	Desmosterol	—	—	—	0.04	—
	100 D:C	—	—	—	1.7	—
4th	Cholesterol	1.5	—	—	—	—
	Desmosterol	0.03	—	—	—	—
	100 D:C	2.0	—	—	—	—
5th	Cholesterol	3.9	3.3	—	—	2.8
	Desmosterol	0.09	0.02	—	—	0.05
	100 D:C	2.3	0.6	—	—	1.8
6th	Cholesterol	—	—	—	0.7	—
	Desmosterol	—	—	—	Not detectable	—
	100 D:C	—	—	—	—	—
7th	Cholesterol	—	2.6	4.1	—	2.8
	Desmosterol	—	0.03	0.07	—	0.03
	100 D:C	—	1.1	1.7	—	1.1
8th	Cholesterol	—	2.7	5.2	—	2.8
	Desmosterol	—	0.02	0.17	—	0.04
	100 D:C	—	0.7	3.3	—	1.4

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Polyamines and Brain Tumors[1,2]

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ABSTRACT—Although their precise functions are unknown, the polyamines have been linked to cellular proliferation and division. The demonstration of a definite correlation between growth rate and the synthesis of the polyamines in tissues has led to the hope that such correlations might extend to polyamine concentrations in physiologic fluids. Polyamine determinations may then offer a simple index of tumor burden and growth rate. Studies of the relationship of cerebrospinal fluid polyamine levels to the presence of brain tumors indicated that these determinations may assist in the early diagnosis of brain tumors, in the long-term evaluation of tumor therapy, and in the short-term evaluation of the efficacy of specific therapies.—Natl Cancer Inst Monogr 46: 127–131, 1977.

Although biochemists have known about the polyamines spermidine ($NH_2(CH_2)_3NH(CH_2)_4NH_2$) and spermine ($NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$), and their precursor putrescine ($NH_2(CH_2)_4NH_2$) for many years, scientists have only recently become interested in their metabolism and function. During the past decade, a series of reviews (*1–8*) have dealt with many of the known aspects of polyamine metabolism and their possible function. Although their precise function has not been determined, in vitro and in vivo studies have linked the polyamines to the metabolism, structure, and function of the nucleic acids (*2, 3, 6*) and thus to cellular proliferation and division (*9*).

Four enzymes are responsible for the formation of the polyamines in mammalian cells (*8, 10*). L-Ornithine decarboxylase catalyzes the conversion of L-ornithine into putrescine; *S*-adenosyl-L-methionine decarboxylase catalyzes the conversion of *S*-adenosyl-L-methionine into 5′-deoxy-5′-*S*-(3-methylthiopropylamine) sulfonium adenosine; spermidine synthase catalyzes the transfer of a propylamino group from 5′-deoxy-5′-*S*-(3-methylthiopropylamine) sulfonium adenosine to putrescine to produce spermidine; and spermine synthase, in a smiliar reaction, catalyzes the transfer of a propylamine moiety from the same substrate to spermidine to produce spermine.

After action by various growth stimuli and in rapid growth systems, the enzymes in the polyamine biosynthetic pathway become more active, and the polyamines subsequently accumulate in vitro and in vivo (*7, 11*). In a variety of tissues, dramatically elevated levels of ornithine decarboxylase (*4, 6*) represent an apparent priming for growth. In contrast, the ornithine decarboxylase activity in quiescent and nondividing cells is low.

Studies of the Ehrlich ascites carcinoma in mice (*12, 13*) have shown that the deceleration in growth rate associated with an increase in tumor size was accompanied by a decrease in ornithine decarboxylase activity and a decline in the concentration of putrescine. Based on these data, Woo and Simon (*14*) formulated a mathematical model to express tumor burden; this model described the relationship observed between putrescine concentration and the growth pattern of the Ehrlich ascites tumor.

The activity of ornithine decarboxylase in rat hepatomas is enhanced with increasing growth rate (*15*), whereas the activities of ornithine transaminase in glutamate synthesis (*16*) and ornithine carbamyltransferase in citrulline synthesis (*17*) decrease. Weber et al. (*17*) have suggested that the increased utilization of ornithine for polyamine synthesis may confer on the hepatoma cells a selective advantage that may contribute to their high growth rate.

To investigate further the relationship of polyamine metabolism to cellular growth rate, we measured the activities of ornithine decarboxylase, *S*-adenosylmethionine decarboxylase, and the levels of the polyamines in a rat brain tumor grown in culture and correlated these levels with the continuous changes in specific growth rate (*9, 18*). A high positive correlation was demonstrated

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between the levels of spermidine, the activities of both enzymes, and the changes in specific growth rate. This high correlation between specific growth rate and the synthesis of the polyamines indicated that these events are associated primarily with cell replication. Extending such correlations and mathematical formulations to polyamine concentrations in physiologic fluids as a reflection of tumor burden or growth rate is of particular interest, and a number of studies are being conducted to verify this hypothesis.

POLYAMINES AS CANCER MARKERS

In 1971, preliminary data were published that showed the possible relationship of elevated urinary levels of polyamines to the presence of human cancers (*19*). These initial studies involved the use of high-voltage paper electrophoresis; this methodology was not particularly suited to the analysis of polyamines in physiologic fluids. Its sensitivity was limited, and the method did not separate the polyamines completely from other substances. Since that time, considerable effort has been expended on refining analytical techniques and, although a totally suitable method has not been devised, current techniques permit us to conduct clinical investigations. Methods now available include gas–liquid chromatography (*20, 21*), gas-chromatography–mass spectrometry (*22*), thin-layer chromatography (*23, 24*), enzymatic assay (*25*), high-pressure liquid chromatography (*26*), ion-exchange chromatography on the amino acid analyzer (*27–31*), and the newly emerging radioimmunoassay (*32*). We use a Durrum D-500 amino acid analyzer, a method that provides good sensitivity and keeps sample handling to a minimum but is too expensive for many laboratories.

Numerous preliminary studies show that polyamine levels are elevated not only in the urine but also in the sera (*27, 32, 33*) and cerebrospinal fluid (CSF) (*34, 35*) of many patients bearing tumors. Many tumor types were investigated, but for the most part, large numbers of cases of any one type have not been accrued. Recently, we studied 79 patients with malignant melanoma classified according to clinical stage and activity of disease (*36*) and demonstrated a correlation between frequency of urinary polyamine elevation and disease activity. We found that, at least for patients with malignant melanoma, a single polyamine frequently was elevated in those whose disease was considered stable; elevation of two or more polyamines was needed for a reliable index of tumor activity. On the other hand, the results of our studies on the elevations of CSF polyamine levels in patients with brain tumors do not indicate that multiple elevations are required for reliability in this fluid.

Apparently, each tumor must be studied in detail because different tumors seem to have different patterns of polyamine elevations. In addition, various other causes such as pregnancy and certain hypermetabolic states may provoke nonspecific (to tumor) polyamine elevations. Some evidence exists to indicate that oral contraceptives might elevate polyamine levels (*36*), and from preliminary data, we know that polyamine levels vary during the menstrual cycle. All this indicates that much more investigation must be done before we can accept these increases as reliable diagnostic markers. However, their value in monitoring therapy (this may be their most important function) in patients with known cancer is growing.

POLYAMINES AND BRAIN TUMORS

A CSF tumor marker would be particularly valuable inasmuch as the primary diagnosis of brain tumors and the evaluation of brain tumor therapy may be both difficult and associated with procedural risk. If such a marker were sufficiently sensitive to predict a decline in clinical status or a favorable response to therapy before present methods, it might be critical to the eventual success of brain tumor therapy.

The measurement of the small quantities of polyamines present in CSF posed a difficult problem. The development of effective methods had been somewhat slow and consumed considerable time. Some aspects are critical in the available assays, and the data must be carefully evaluated. Nevertheless, these approaches have generated valuable data.

Table 1 shows the results of CSF polyamine analyses in a reference group made up of patients with various neurologic diseases but without brain tumors (*35*) and in groups of patients with untreated brain tumors. The reference group was not comprised of normal healthy volunteers (though such a group is being studied), and hence it should be considered only for comparative purposes.

The CSF putrescine concentrations of patients with glioblastomas and medulloblastomas were significantly different from those in the reference group. We noted some overlap between the astrocytoma group and the reference group, but the difference was still significant. The patients with meningioma and metastatic tumors were more

TABLE 1.—*CSF polyamine levels in reference and brain tumor groups*

Diagnostic group	No. of patients	CSF polyamines, pmoles/ml[a]	
		Putrescine	Spermidine
Reference	29	184±54 (92–278)	150±48 (97–293)
Glioblastoma	11	546±89 (460–771)	285±40 (221–338)
Medulloblastoma	16	561±164 (350–889)	331±101 (201–543)
Astrocytoma	19	365±118 (125–577)	290±139 (122–546)
Meningioma	7	265±55 (184–344)	381±167 (173–649)
Pituitary adenoma	19	248±90 (95–489)	195±83 (84–356)
Metastatic tumor	8	301±126 (122–454)	293±127 (161–564)

[a] *Values* are mean±SD with range *in parentheses.*

TABLE 2.—*Comparison of polyamine levels in treated and clinically stable patients with those of untreated patients*

Diagnostic group	No. of patients	CSF polyamines, pmoles/ml[a]		Significance vs. untreated group[b]	
		Putrescine	Spermidine	Putrescine	Spermidine
Glioblastoma	7	298±74 (172–395)	217±71 (106–341)	<0.001	<0.05
Medulloblastoma	16	307±110 (132–596)	205±69 (122–341)	<0.001	<0.001
Astrocytoma	20	277±105 (161–531)	215±56 (109–307)	<0.05	<0.01

[a] *Values* are mean±SD with range *in parentheses.*
[b] *Values* are results of *t*-test for unpaired groups. *See* table 1.

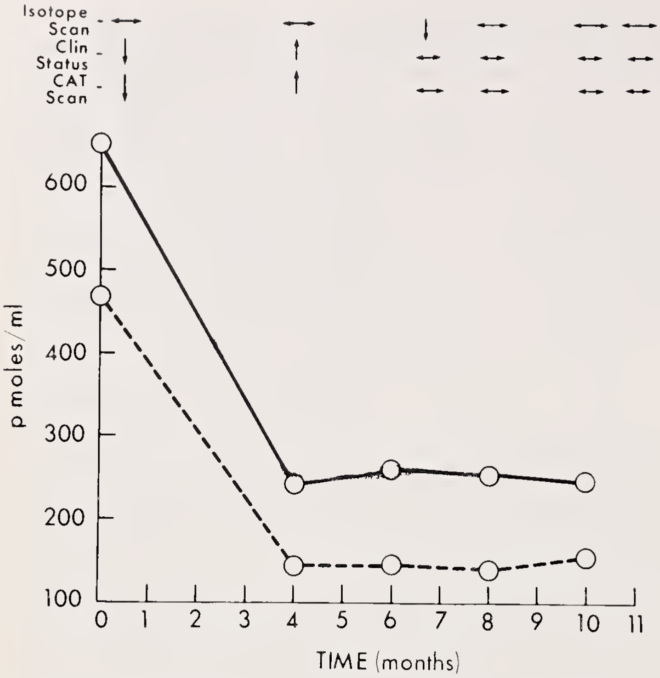
variable, and many of the values for those with a pituitary adenoma were within the reference range. Although the spermidine concentrations were generally similar to those for putrescine, the differences were less marked. Meningiomas are an exception because the increase in spermidine concentrations in these patients was greater than that for putrescine. Spermine was not detected in the reference group and was noted only infrequently in the tumor groups.

When compared with concentrations found in patients without tumor, those with untreated malignant central nervous system tumors have elevated CSF polyamine concentrations. However, polyamine increase can be fairly nonspecific (to tumor), and our preliminary data indicate that cerebrovascular disease, certain degenerative diseases, and infection may also raise the levels of the polyamines in the CSF. These increases do not appear to be as great as those caused by malignant brain tumors, but other disease entities must be borne in mind. Samples containing blood also tend to be elevated. Polyamine concentrations in CSF bear no particular relationship to CSF protein and therefore are not merely reflective of alterations in the blood–brain barrier.

Though polyamine determinations have potential for early diagnostic purposes, perhaps even more important is their potential for monitoring brain tumor therapy. Table 2 lists the results of CSF polyamine analyses on groups of patients with malignant brain tumors who were undergoing chemotherapy and showed definite clinical evidence of tumor regression at the time the CSF specimens were obtained. A comparison of these groups with their untreated counterparts shows that there are statistically significant differences in the untreated versus the treated groups, the levels in the treated groups approximating the levels in the reference group. On the basis of these observations, it appears that polyamine values relate to tumor burden and/or tumor growth rate.

Serial CSF polyamine analyses performed for a period of months on a number of patients undergoing chemotherapy for brain tumors reveal an apparent relationship between the status of the patient and polyamine levels. In several patients, significant increases (particularly in putrescine concentrations) precede a decline in clinical status due to tumor regrowth. The reverse is also seen; i.e., polyamine levels decline with improvements in clinical status. Text-figure 1 shows the results of serial polyamine determinations performed during an 11-month period on a patient with a medulloblastoma. It also depicts changes noted in the isotope scan, the computerized axial tomographic (CAT) scan, and the clinical status as evaluated by neurologic examination. The first (preoperative) sample showed significant increases in the levels of putrescine and spermidine. The patient's clinical status was declining at this time, and the CAT scan revealed a lesion, although the isotope scan was negative. After operation, he was treated with radiation followed by chemotherapy. He improved significantly for a while and has since maintained the status quo.

CSF polyamine determinations may also provide a rapid evaluation of the efficacy of a specific course of therapy. We followed a selected group of patients with multiple lumbar punctures during the first week after chemotherapy. For various reasons, not one of these studies has been completed, but our preliminary observations show a significant rise in the CSF putrescine concentration 4–6 days after treatment in patients harboring responsive tumors. Text-figure 2 presents the partial results obtained in 2 such studies on a patient who had an inoperable thalamic tumor and was started on chemotherapy. The *solid line*



Text-figure 1.—CSF polyamine levels and changes in isotope scan, CAT scan, and clinical status for 11 mo in a patient with a medulloblastoma. —— = putrescine concentration; – – – = spermidine concentration; ⟷ = no change from previous evaluation; ↑ = improved since previous evaluation; ↓ = declined since previous evaluation.

indicates the changes in the putrescine concentration during the first course of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) therapy; a significant increase was noted 96 hours after therapy began. Unfortunately, no samples were available in the period bracketing 96 hours, so we assume that the maximum rise occurred then. After this course of therapy, the patient responded well and improved significantly. The *broken line* indicates the changes seen in the putrescine concentration during the second course of BCNU therapy; no significant rise was observed in the 120 hours studied. After this course of therapy, the patient's clinical status declined, and he died several months later.

Thus CSF polyamine determinations show promise as aids in the early diagnosis of brain tumors, in the long-term evaluation of treatment, and in the short-term evaluation of the efficacy of a specific course of therapy.

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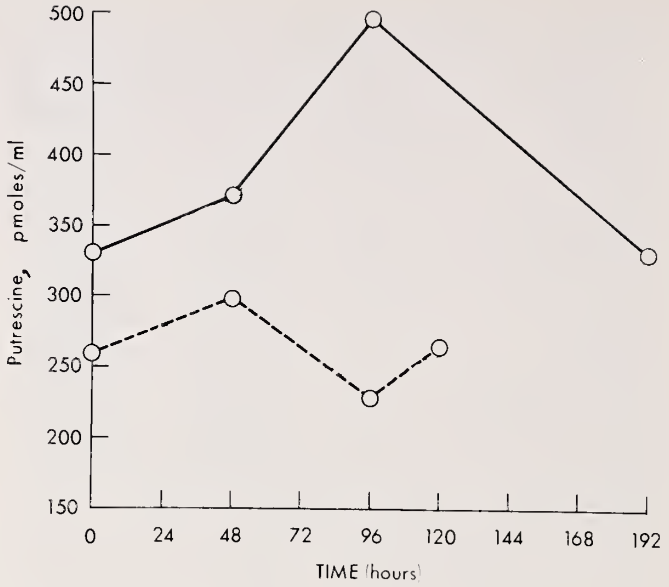

Text-figure 2.—Changes in CSF putrescine concentration in the same patient during two courses of chemotherapy. The 0-hour samples were obtained just prior to the initiation of therapy. —— = first course; – – – – = second course.

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Astrocytin and Malignin: Two Polypeptide Fragments (Recognins) Related to Brain Tumor[1,2]

Samuel Bogoch[3,4]

ABSTRACT—The first cancer antigen-like compounds of known constant composition have been produced: from human malignant brain tumors, astrocytin (approximate mol wt 10,000; composition: aspartic acid 9, threonine 5, serine 6, glutamic acid 13, proline 4, glycine 6, alanine 9, valine 4, 1/2 cysteine 2, methionine 1, isoleucine 2, leucine 8, tyrosine 2, phenylalanine 3, lysine 8, histidine 2, arginine 4, total 88), and from malignant glioma cell culture fermentation, malignin (approximate mol wt 10,000; composition: aspartic acid 9, threonine 5, serine 5, glutamic acid 13, proline 4, glycine 6, alanine 7, valine 6, 1/2 cysteine 1, methionine 2, isoleucine 4, leucine 8, tyrosine 3, phenylalanine 3, lysine 6, histidine 2, arginine 5, total 89). Antibodies to one react identically with the other compound. Topographic antigen-like reagent templates (TARGET reagents) have been synthesized from those compounds, which permit the production from serum of specific antibody-like fragments [TARGET-attaching globulin(s) (TAG)], the quantitative determination of which provides a serum diagnostic test for brain tumor (92% accurate, 4% possible false positives). In addition, a TAG immunofluorescent stain provides a tissue diagnostic test. TAG are cytotoxic to malignant glial cells in vitro. We are now applying the same technology to other cancers. The structures of astrocytin and malignin, unique by computer search, relate to primitive anaerobic respiratory enzymes. This observation may be a fundamental new clue to understanding the survival efficiency and dominance of the cancer cell, and the compounds produced herein may represent potential new cancer therapies.—Natl Cancer Inst Monogr 46: 133–137, 1977.

We have been studying the gangliosides and the glycoproteins of the nervous system in terms of their structure, localization, and function (*1–3*). As a result, we proposed that these substances are involved in high information states and recognition functions. One test of this hypothesis is to examine these compounds in low information states. Evidence has been found that cancer cells are in a low information state, in the sense that cell division proceeds regardless of the fact that the space available has been used up (failure of contact inhibition) and proceeds to the death of the host and hence of the cancer cells.

After 12 years of studying brain cancers in various in vivo and in vitro states, the following results can be reported (*4, 5*):

We isolated astrocytin, the first cancer antigen-like compound of known constant composition from human malignant brain tumors. New compounds, topographic antigen-like reagent templates (TARGET reagents), have been synthesized that contain the immunochemical specificity of the in situ cancer antigen. By using these TARGET reagents, we isolated pure, specific antibody-like material(s) [TARGET-attaching globulin(s) (TAG)] from human and animal serum. The quantitative measure of serum TAG has provided the first quantitative serum diagnostic method for malignant brain tumors. We demonstrated that the TAG compounds react with the cancer antigen-like compounds in quantitative precipitin, Ouchterlony diffusion, and double-layer immunofluorescence. The last-named reaction constitutes a new tissue diagnostic method for malignant glia.

The cancer antigen-like material has now been produced by glioma cell-culture fermentation. This product (malignin) is structurally unique from astrocytin but shares its immunochemical properties. The bulk production of malignin permits the large-scale use of TARGET reagent in diagnostic testing. That the percentage of extractable cancer-cell protein that is malignin rises as the malignancy of the cancer growth increases has been demonstrated. A computer search of the library of protein structures revealed that astrocytin and malignin have unique structures: only four are even remotely related to them and each is a respiratory protein. This finding may be of great significance because it may provide the first biochemical insight into the basis of the anaerobic respiratory activity of cancer cells and

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Supported by funds from Brain Research, Inc., New York, N.Y.

[3] Boston University School of Medicine, and the Foundation for Research on the Nervous System, 36 The Fenway, Boston, Mass. 02215.

[4] The author is grateful to his colleagues who provided serum samples and tumor and brain tissue specimens, who examined the specimens by immunofluorescent microscopy, and to those who provided technical assistance.

their dominance. It may also provide new therapeutic means for their destruction because TAG compounds were highly cytotoxic to malignant glia in vitro. The implications of these findings extend beyond cancer diagnosis and treatment into tissue and organ specificity, transplantation rejection, specific pharmacologic delivery systems, and developmental disorders. For example, a third recognition substance of this type that is of abnormal molecular weight has been identified in Reeler disease in mouse brain. With the production of this third product, the family name "recognins" has been proposed for the recognition substances and the name "chemoreciprocals" for their antibody-like counterparts.

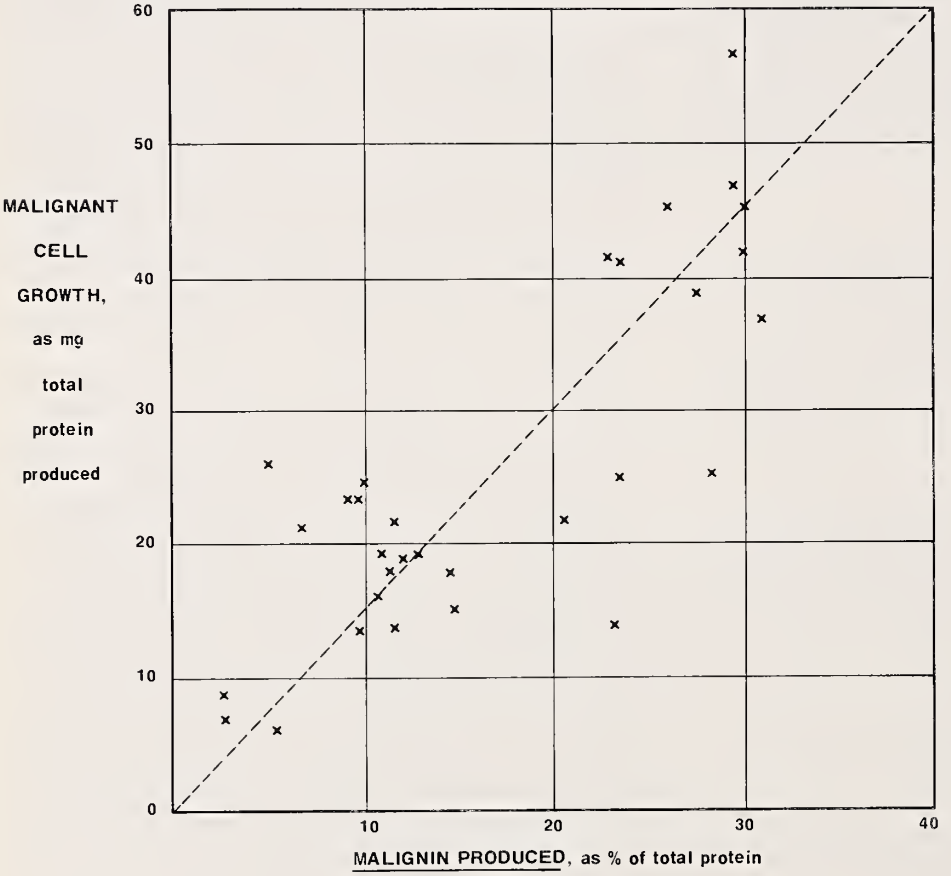
THE STRUCTURE OF ASTROCYTIN AND MALIGNIN

The two unique, low-molecular weight protein fragments named astrocytin and malignin are produced from in vivo brain glial tumor and in vitro cancer cell fermentation, respectively. They bear specific tissue antigen and other immunochemical properties characteristic of the tumor.

Astrocytin has a molecular weight of approximately 8,000 by thin-layer chromatography (9,690 by calculation) and the following approximate composition: aspartic acid 9, threonine 5, serine 6, glutamic acid 13, proline 4, glycine 6, alanine 9, valine 4, 1/2 cysteine 2, methionine 1, isoleucine 2, leucine 8, tyrosine 2, phenylalanine 3, lysine 8, histidine 2, arginine 4, total 88.

Malignin, repeatedly produced over 80 generations from glioma cell-culture fermentation, has a molecular weight of approximately 10,000 (approximately 10,150 by thin-layer gel chromatography, and 10,067 by calculation) and the following approximate composition: aspartic acid 9, threonine 5, serine 5, glutamic acid 13, proline 4, glycine 6, alanine 7, valine 6, 1/2 cysteine 1, methionine 2, isoleucine 4, leucine 8, tyrosine 3, phenylalanine 3, lysine 6, histidine 2, arginine 5, total 89.

The percentage of total extracted cancer cell



Text-figure 1.—Increase in malignin produced, as percentage of total protein, with increase in malignant cell growth.

protein, which is malignin, increases with the degree of malignancy of the growth (text-fig. 1).

ANTIBODIES TO ASTROCYTIN AND MALIGNIN

Antibodies prepared in rabbits to astrocytin are specific to both astrocytin and malignin, react with them in quantitative precipitin and Ouchterlony double-diffusion reactions, and label in situ glial cancer cells specifically in double-layer immunofluorescence (hence a new tissue diagnostic method).

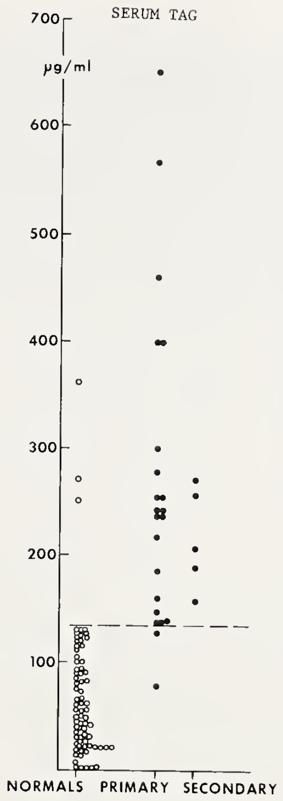
Figure 1 shows a section of brain removed at surgery from a patient with a malignant glial tumor. The section is stained with the new product TAG, an antibody-like protein fragment prepared from rabbits challenged with the recognin and with fluorescent goat anti-rabbit serum. Only tumor glia stain; normal glia, neurons, blood vessels, and other structures do not.

This new tissue diagnostic test promises to be useful: 1) in identifying the presence of a malignant tumor (vs. a benign one) at surgery, and 2) in defining presence of malignant glia in the edges of the tissue removed at surgery and indicating whether all of the tumor was removed.

Since TAG compounds also are highly cytotoxic to these cancer cells in vitro, their therapeutic potential requires exploration in man.

SERUM TAG TEST FOR MALIGNANT BRAIN TUMORS

Synthesis of astrocytin or malignin complexes with carriers such as cellulose derivatives produces solid TARGET. These reagents react with antibody-like globulins in human serum and from the resulting complexes, TAG can be isolated. Text-figure 2 shows the quantity of serum TAG in micrograms of protein per milliliter for normals and tumors. TAG is 0–130 in 71, greater than 130 in 3 (mean 54.3) in normals and nontumor medical and surgical disorders; 137–650 in 20, less than 130 in 2 (mean 246.4) in malignant brain tumors; 157–270 in 5 secondary malignant tumors in brain; 288 and 442 in 2 cancers of the prostate with no apparent brain secondaries; 31 and 36 in 2 sarcomas with no apparent secondaries to brain; and 9 with as yet uncertain clinical diagnoses. The above data represent two successive blind studies with a total of 114 serum specimens and support the utility of the serum TAG determination as a diagnostic procedure for malignant brain tumors. The serum TAG diagnosis of primary malignant brain tumors in these studies is 91% accurate; of primary plus secondary, it is 92.6% accurate. There may be 4% false positives in the combined normal and medical–surgical disorder group. There were no false positives in normal symptom-free individuals. The group of malignant brain tumors is distinguished from the normal and medical–surgical disorder group at a level of $P < 0.001$.



TEXT-FIGURE 2.—Serum TAG determinations of primary and secondary malignant brain tumors.

STRUCTURAL RELATION TO RESPIRATORY PROTEINS

Computer search confirms the unique structure of astrocytin and malignin. The closest structures are certain respiratory proteins (table 1). If the in situ equivalents of the cancer recognins prove to have respiratory functions in cancer cells (since they account for such a large proportion of the extracted cell protein), we may have identified a biochemical mechanism that for the first time explains the voracious respiratory activity[5] of the cancer cell. This might also explain the absolute

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[5] *Note* especially their anaerobic properties; as Warburg showed, neoplastic cells can survive for long periods in the absence of glucose and oxygen, even in the presence of cyanide.

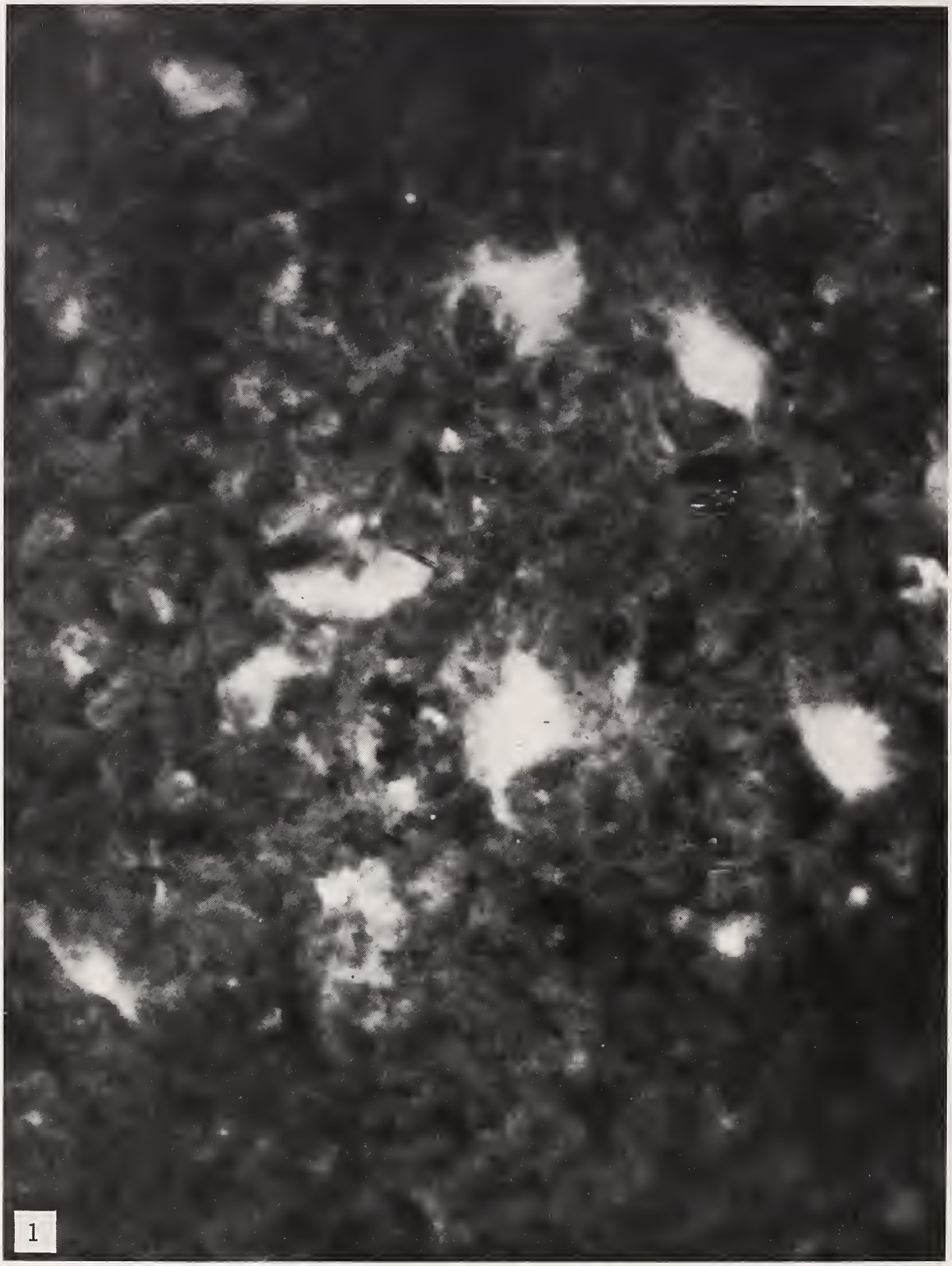

FIGURE 1.—Malignant glial cells in human brain tumor stained in immunofluorescence with TAG.

TABLE 1.—*Comparison of the structures of astrocytin and malignin with nearest structures by computer search*[a]

Amino acids	Astrocytin	Malignin	Cyto-chrome b_5[a]	Ferredoxin		Acyl carrier	Neurophysin		Gonado-tropin release
				Leucaena alauca[a]	Alfalfa[a]	*Escherichia coli*[a]	Bovine	Pig	
Aspartic acid	9	9	9	10	8	7	2	3	0
Threonine	5	5	6	4	6	6	2	2	0
Serine	6	5	5	7	8	3	6	7	1
Glutamic acid	13	13	14	13	13	14	9	9	0
Proline	4	4	3	5	3	1	8	7	1
Glycine	6	6	6	7	7	4	16	14	2
Alanine	9	7	4	6	9	7	6	7	0
Valine	4	6	4	6	9	7	4	2	0
½ Cysteine	2	1	0	5	5	0	14	14	0
Methionine	1	2	1	0	0	1	1	1	0
Isoleucine	2	4	4	4	4	7	2	2	0
Leucine	8	8	7	10	6	5	6	7	1
Tyrosine	2	3	3	3	4	1	1	1	1
Phenylalanine	3	3	3	3	2	2	3	3	0
Lysine	8	6	7	5	5	4	2	2	0
Histidine	2	2	7	1	2	1	0	0	1
Arginine	4	5	3	2	1	1	7	5	1
Asparagine	0	0	0	0	1	2	3	2	0
Tryptophan	0	0	1	1	1	0	0	0	1
Glutamine	0	0	0	4	3	4	5	4	0
Total No. of residues	88	89	87	96	97	77	97	92	10
Molecular weight[b]	8,000[b]	10,000[b]	10,035	10,588	10,483	8,509	10,065	9,488	1,201

[a] Only four close structures were found; other three are shown for comparison.

[b] Weights were determined by thin-layer gel chromatography, calculated molecular weight of above structures: astrocytin 9,690 and malignin 10,067.

rise in malignin with increased malignancy (text-fig. 1) and why TAG is cytotoxic to cancer cells (it binds an essential respiratory protein).

DISCUSSION

Our earlier definition of recognition substances in mature brain cells that operate during high information states of memory and learning led to the observation that these substances are replaced in cancer by in situ compounds that have the immunochemical specificities of astrocytin and malignin. These new compounds characterize the low information state of the cancer cell, in that cell division proceeds to the death of the host, and hence of the cancer cells. With the production of these first compounds of the group, the family name of recognins is proposed. For the antibody-like compounds to the recognins, the name chemoreciprocals is proposed.

The recognins have immediate utility in the diagnosis of brain tumors and possibly others and represent a major advance in our understanding of the structural basis of cancer antigens. We are applying the same technology to other cancers. Study of the recognins may provide new insight simultaneously into the respiratory survival efficacy of cancer cells, and suggest new therapeutic means to destroy them.

Also, these findings are clearly relevant to other tissue and organ specificity studies as in transplantation rejection, to disease specificity, and to means of specific delivery of pharmacologic agents to particular subcellular and cellular addresses. They also have clear applications to defining and treating developmental disturbances such as those of cell positioning. For example, we have now isolated a third recognin, that in Reeler disease of mouse brain. In this genetic movement disorder in which particular groups of nerve cells fail to migrate to their normal position, Reeler recognin is obviously abnormal, since it has a molecular weight of 3,600–5,000, compared with 8,000 in normal mouse brain (*5*).

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(*4*) ———: Malignin: A polypeptide related to brain malignancy. Trans Am Soc for Neurochem 7, No. 1:239, 1976

(*5*) ———: Brain glycoproteins and recognition functions: Recognins and cancer. *In* Recent Advances in Sphingolipidoses and Allied Disorders (Volk B, ed). New York: Plenum Press, 1976, pp 555–566

DISCUSSION III

G. D'Angio: Dr. Kricheff, will you describe the method you use for image enhancement? What particular substance do you use, and how do you go about it?

I. Kricheff: Any contrast enhancement material is adequate as long as it is an intravenous or intra-arterial iodine compound, e.g., Conray (Mallinckrodt), Renografin (Squibb), and the Hypaque series (Winthrop). Some have suggested that the nonsodium agents produce a little more enhancement. Almost everyone is giving 28–40 g iodine, and around 30 g is standard. It can be delivered by giving 200 cm^3 of 30% solution, as we do; or 100 cm^3 of 60% solution, as some of my colleagues do; or a lesser amount of a higher concentration. It does not matter how the agent is delivered as long as it is done in less than 6 minutes and a high blood level is achieved that then enters the extracellular space. Some inject it by a bolus. We use a drip because we believe that is more efficient and is associated with a lower incidence of nausea and vomiting.

Two EMI units, one a head and the other a body unit, are operational. The body unit gives magnificent head pictures, but it does have a high signal-to-noise ratio, and apparently involves a high radiation dosage. McCullough, who has measured the dosage, believes it varies from 2 to 12 rads/slice, whereas others believe it is below 2.

The Delta scanner on the market does a nice job. Phillips is promising a scanner. I do not believe General Electric has yet delivered (February 1976) its fan-beam unit to the University of California, and many people here and in Europe believe that fan-beam technology attempts too great a leap forward. Picker has advertised a unit, but people at the Cleveland Clinic tell me it has yet to make any images. General Electric is marketing a head unit that is similar to the original EMI. Syntex has a unit and showed some good pictures recently. Pfizer is marketing an Acta-scanner, which was the first on the market to do the whole body and to scan through air. Phillips has a team of about 30 scientists working here and many more in Europe. General Electric has a group of 49 scientists working in Schenectady, but nobody is talking about the problems they are encountering. If I had to buy a scanner now, I would choose the EMI body scanner because it gets good pictures of all parts of the body in the shortest time. EMI has the longest experience in the field, and even though its computation time is rather long, it will be reduced within the next 6 months or a year.

M. S. Mahaley: With regard to the ventricular dilation found in the CAT scan after radiotherapy, Wilson[1] described a series of patients in whom he and his colleagues did pneumoencephalography after radiotherapy. They concluded that ventricular dilation was a consequence of the radiotherapy. Would the panel members comment on two points: First, the problem of distinguishing tumor recurrence from radiotherapy changes, and the relative value of the isotopic and CAT scans in separating these two entities in the patient who has received radiotherapy and who is being followed after treatment for a glioma. Second, when we see no enhancement, even with contrast procedures, in an area of low density that was also the site of operation, how can we be certain that there is no tumor there? We appreciate that enhancement can help to identify the presence of tumor, but how about the opposite?

M. Cooper: The radiopharmaceuticals are nonspecific and the standard radionuclide scan can do little to distinguish the effects of radiotherapy from tumor regrowth. Studies using radioammonia or some similar approach may help. The difficulty of distinguishing postsurgical intervention from tumor regrowth is a real one, and we are probably working at the limits of present technology, even with the EMI. In any event, standard radionuclides do not help to distinguish tumor regrowth from radiotherapy or other reactions around the surgical site.

Kricheff: I do not think enough data have been accumulated with long enough follow-up to allow us to say how accurate the CAT scan is. On the positive side, if a contrast injection is given and an area lights up, one can say, "Yes, this is tumor. It's not radiation effect; it's recurrent tumor." On the negative side, we do not have much information. For example, we are concerned that steroids will block the contrast enhancement effect and give rise to false negatives. We just do not know what effect drugs will have. It is the best technique available. It is better than nuclear scan because you do not have the problem of the bone flap and postoperative changes, but we do not have the objective evidence that would permit us to say that CAT is 90% accurate, 40% accurate, or what have you.

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[1] Wilson GH, Byfield, J, Hanafee WN: Atrophy following radiation therapy for central nervous system neoplasms. Acta Radiol 11:361–368, 1972.

We have a long way to go in CAT technology both in the mathematics and in the detector aspects. Sector scans will allow us to concentrate the data, analogous to rotational radiation therapy. This would concentrate data accumulation in the area of interest and produce considerably more data, giving us the ability to get thinner "slices," i.e., more specific information in a particular area.

J. Ransohoff: Is it not true that the gallium scan is better than the technetium in postoperative patients because pick-up in the postoperative bone defect, if not absent, is much less trouble? I believe that for purposes of postoperative follow-up, the gallium scan plus a CAT is more effective than one technique alone.

R. Blasberg: What we are really looking for with the nuclide and the enhancement CAT scans is a defect in the blood–brain barrier. With a malignant tumor, when enhancement or a positive uptake with the nuclide scan is seen, the existence of such a defect is being documented. Changes in the scan image may lead one to conclude that the tumor is getting smaller or that there is some other improvement, but what you are saying is that the barrier is becoming less leaky. Have any of the panel members noted or seen documented differences between low-grade astrocytomas and the malignant forms, in terms of CAT enhancement and standard nuclide scans?

D'Angio: Dr. Blasberg, do you think tumors that seem to decrease in size on scans are necessarily smaller?

Blasberg: Without enhancement, one can get information on other parameters, particularly from the CAT scan, in terms of mass effect and differences in density; when we are talking about enhancement or the standard nuclide scan, we are seeing defects in the barrier. When improvement is noted in those studies, it is also seen in barrier function, and we should recognize that. To say that these studies are improving does not necessarily mean that we are getting a tumor response, although Dr. Wilson has shown that a close correlation exists between at least the nuclide scans and the clinical status. With these studies, we are actually observing improvement of the barrier function.

Cooper: I have seen that frequently. When steroids are given the scan becomes negative; and I am assuming that this represents some steroidal effect on the blood–brain barrier. I have no experience with transverse tomography, but I agree that with contrast agents and with radionuclides, we are looking at a blood–brain barrier effect.

Kricheff: Other parameters are present besides contrast enhancement. In the near future, there will be numerous publications on detailed anatomic analysis, e.g., those concerning subtle changes in the cisterns, the kind of thing we used to look for in encephalograms. One of the great advantages of the CAT scan is that in addition to looking for enhancement, one can seek mass effects and changes in the ventricles.

D'Angio: What about less malignant tumors?

Kricheff: A couple of months ago, I started looking at our gliomas to see if a significant difference occurred in the degree of enhancement between those that were severely malignant and those that were moderately so. I did not find enough that were reported as minimal anaplasia. A random distribution was noted of the ones our pathologists reported as moderate and those they reported as marked anaplasia. I am glad Dr. Blasberg asked that question. I have not seen a difference, but this was a first "go-through." I looked at the extent of edema and whether any ring effects were present and found a random distribution of diffuse contrast enhancement and a small amount of edema. I just was not able to find any correlation in about 15 parameters.

O. Salazar: Apparently, we have good diagnostic methods for some of the brain tumors. How accurately do we determine true tumor extent in the more malignant glioblastoma multiforme? This is of particular importance for the radiation therapist who is going to give irradiation after surgery. A considerable brain shift is seen after extensive resection, and the edema that accompanies the tumor and is present after the operation is produced by radiation therapy. In the past, surgeons have put in tantalum seeds to guide our portals. When we give whole-brain irradiation, this is not important, but when we are giving a supplemental dose to the primary site, we have to define the target volume. What use do you see here for CAT scans? We have done serial brain scans in some patients, but the flap effect after operation makes this extremely difficult.

Cooper: I am not optimistic when using radionuclide scans. We need multiple isotope studies. We can use bone-seeking agents to identify the flap, gallium or the pyrophosphates to delineate tumor necrosis or the specific tumor, and the pertechnetate scan to identify the whole lesion.

We can also use stereoscopy by viewing scans taken at 10-degree intervals. I am not aware, however, that these have produced results that would help to plan radiotherapy beams. The CAT scanner probably offers a better chance. If we can look at the absorption coefficients of the different types of tissue within the lesion, and perhaps by using three-dimensional reconstruction, we may be able to distinguish the changes in absorption in edema versus tumor or scar tissue.

Kricheff: The CAT scan offers the therapist the view he likes to reconstruct in his head. Most workers believe that if enhancement is noted, you are seeing the tumor, but we have no proof of that. We accept that that is the extent of the tumor because we see it. Again, no objective studies have been published because the technology is too new and documentation in an objective way is difficult. How do you interpret an assertion that "Yes, the CAT showed us exactly where to place our portals"? By the time you get inside or the patient has expired, conditions have changed a great deal. We are saying that if it enhances well and the steroids have not blocked the enhancement, then that is the tumor and the rest is the edema.

D. Rothner: A recent modification of the Delta scanner, called a "joy stick," consists of a small circle that can be moved around the screen. When it is centered on an area, one gets an immediate reading with standard deviation of absorption coefficients, so that one can compare immediately areas at the site of and peripheral to the lesion; e.g., in liver and in brain one can identify a nodule. However, such comparisons have not been quantitated in terms of pathology.

K. Charyulu: Dr. Kricheff showed a slide from a patient with a craniopharyngioma. The tumor was removed and edema developed later in the temporoparietal area, more posteriorly, and maybe laterally. What portals were used in that patient? Was the whole brain treated, or was a small portal directed to the craniopharyngioma?

Ransohoff: That patient was treated for a craniopharyngioma, and an infarct developed on the side opposite to the operative site. A beam from a linear accelerator was directed at the tumor.

Charyulu: Is it not customary to use a small portal for craniopharyngioma?

Kricheff: Treatment was given with a small portal. The changes seen on the scan initially were secondary to vascular occlusions in the region of the circle of Willis. Angiography demonstrated multiple middle-cerebral branch occlusions at the base. We believe this to have been a radiation effect leading to vasculitis. The scan showed areas of infarction scattered throughout the brain which, as you know, is one of the explanations for radiation edema and necrosis.

Charyulu: Can radiation produce vasculitis in such a short period? It is part of the tumor, is it not?

Ransohoff: That vascular occlusion may have been secondary to the operation.

D'Angio: We will proceed with a discussion of therapy. Dr. Cooper and Dr. Kricheff, what should the sequence be today in working up a patient with a suspected brain tumor? Does the scan precede the CAT? Where does angiography fit into this? What about air studies?

Kricheff: Both studies are ordered, and either may be scheduled first. The ideal work-up requires the capacity to perform the nuclear or the CAT scan promptly, but this does not happen in many institutions. I believe that everyone gets both studies, and so we have an opportunity to compare the relative efficiency and cost effectiveness of the two.

The CAT scan has the advantage that if it is negative for tumor, it is not just negative. If the patient has an organic mental syndrome and you are looking for a tumor and that patient does not have one, the nuclear scan is read as negative. The CAT scan may be read as cerebral atrophy. In terms of cost and use of available facilities, we must ask: If the CAT scan is positive and clearly outlines the lesion and shows the mass effect, do you need a nuclear scan? I believe you should do a nuclear scan in cases in which, by virtue of its degree of enhancement or of the flow studies, the scan will strengthen the pathologic diagnosis. The main role for the nuclear scan today is when the CAT scan is negative. The nuclear scan always covers the whole head. Under the pressure of work, we have missed lesions when we took four CAT slices when we should have taken five, and did not quite get up to the top of the head. The nuclear scan sees the whole brain and hence has a complementary role in the negative CAT study.

If the CAT scan is negative, one is much more comfortable if the brain scan is also negative.

Cooper: We have not evaluated the complementary role fully. We do comparative studies and immediately go to a crossover to increase our total yield, if one or the other is negative. My impression is that the ease with which the nuclide scan can be done will lead us to keep it as a screening study. Because of the great demand, the CAT scan will be used for more detailed delineations to assist in planning treatment, whether that is surgery or radiotherapy. The introduction of CAT has had a variable effect on the frequency with which brain scans are performed in different centers. We have had the EMI for just over a year, and our brain scans continue to increase at a rate of 35–40% per annum. We are studying different populations, and we must bear this in mind when we try to decide which study to do first. With what sort of population are we dealing? How can we deliver the necessary care most quickly? I believe the examinations are going to end up in some complementary role; precisely how remains to be established.

D'Angio: Dr. Cooper, have we fully explored the kinetics of image development with radionuclide scans? Do we know the best time sequence for developing an image using radionuclides in relation to blood flow patterns in normal tissue?

Cooper: We get a higher positive yield if we delay the standard five-view brain scan for about 2 hours after we have injected technetium pertechnetate. If we use gallium, the wait is considerably longer. With the chelate, technetium diethylenetriamine pentaacetic acid, I think we are able to image within 30 minutes after injection, but we have missed lesions when using chelates for brain tumor scanning. We stick to technetium pertechnetate and scan 2 hours after the injection. Your question raises the matter of blood–brain barrier. When is the optimum time to give the contrast and to do CAT scans? I wonder if the yield would be increased if we waited longer after giving the infusion of contrast material.

S. Jones: I challenge the concept that metastatic lesions should be resected. Patients with cancers of the breast and lung frequently develop metastatic disease in the CNS, and in almost all instances, these can be managed without surgical intervention. If a large group of patients is followed, only a few will benefit from surgical resection. In general, these patients have widespread metastatic diseases and treatment with steroids and whole-brain irradiation controls the CNS manifestations during the remainder of their lives, which usually is not long.

The data Dr. Ransohoff presented suggest that one-third have a single metastatic deposit. The other two-thirds have multiple foci, although many of these may not be detectable by the limited resolution available from current techniques. It seems inappropriate to subject most of these patients to surgical removal of metastatic disease. The occasional patient with melanoma or renal cell carcinoma may have no other evidence of disease, and resection may be appropriate. However, medical oncologists believe that most patients with metastatic cancer should not be subjected to a surgical procedure.

D'Angio: Would you describe your plan for a patient with breast carcinoma who, 3 years later on complete work-up, seems well but the best techniques show a solitary focus in the brain?

Jones: In our chemotherapy series, approximately 10% of our patients who are in good partial or complete clinical remissions have the remission interrupted first by a new CNS relapse. These patients are successfully managed with Decadron and whole-brain irradiation. Only a rare patient has a recurrence of CNS symptoms before some other event. We treat these patients with radiotherapy. If radiation fails to control the disease, or if CNS manifestations persist and no evidence of other metastatic disease is revealed, we might then consider surgical removal.

Ransohoff: It is impossible to talk about "most patients with metastatic brain tumor." The medical oncologist sees these patients at a phase of their clinical evolution that is different from that seen by the neurosurgeon. When you group these patients together, you get a "lump" answer, and I do not like lump answers when I am trying to learn about treating cancer

D'Angio: Dr. Ransohoff, in the patient I have described who has been well and has no other evidence of activation of disease, is the lesion likely to be a second primary?

Ransohoff: The chances of its being a second primary are remote. You will know only when you remove the tumor. A patient who has metastatic tumor in a resectable place and had had a breast cancer removed 3 years before should have that tumor removed. I should have indicated in my paper that all these patients do receive radiation therapy thereafter.

T. Langfitt: I want to emphasize the advantage of steroids in patients who appear to have a single metastatic lesion. In the past, the patient would deteriorate, and one had to decide whether to operate without knowing if only a single lesion existed. Now we can control some of these patients with an apparent single lesion for weeks or even months, and follow them with CAT scanning with enhancement to discover whether they are going to develop a second lesion. If, after a period of several months, the patient still has only one CNS lesion, or the lesion has expanded to the point at which it is threatening life, or the patients has a severe neurologic deficit that can no longer be controlled by corticosteroids, then one is warranted in removing the lesion. In any event, several months have been gained. In the old days, if you had operated on that patient once you found what appeared to be a single lesion, the patient would do well and then a few weeks or months later, he would return with two or three more lesions.

D'Angio: The Chicago group described some patients who did have second primaries. This would never have been known without surgical removal.

L. Rubinstein: Dr. Ransohoff, can you think of any geographical or environmental factor that determines the type of primary tumor in your series or any other series? Your series contained a relatively low proportion of primary tumors in the lung compared, e.g., with that of Wiley McKissick, who found 65% of his brain secondaries to be from a primary in the lung. His series had a high incidence of melanoma. When I was in New York, I also noticed a high incidence of melanomas compared with neurosurgical material described elsewhere.

S. Kramer: Later I am going to report on 2,000 metastatic tumors irradiated in different ways. Approximately 50% are from the lung, about 25% from the breast, and the others are a mixture. The difference in opinion we have just heard reflects experience with patients referred to various departments. Dr. Ransohoff probably does not see patients who have active metastatic disease and are obviously sick. His is a selected group.

What should we do for a woman who has gone 3 years without a recurrence? If she had gone a little longer, I would feel strongly that she had to be investigated and operated on because I had 2 patients with breast cancer who developed meningiomas. It should be emphasized that the primary site is more important than any other factor. If you have a patient with lung cancer and he develops an intracranial mass lesion, it is almost certainly metastatic.

The question of the solitary lesion versus multiple lesions was settled long ago by Galutzy and Payne for lung cancer patients. They studied 750 autopsied cases and found that in less than 10%, the metastases were solitary and in 90% or more they were multiple. Almost all patients with lung cancer have active diseases, and for them surgical intervention is contraindicated except as an emergency measure. There is some indication for surgical intervention when you are dealing with lesions that tend to be solitary and slow growing.

S. Bogoch: Do we have any evidence that the CAT scan allows us to pick up smaller tumors now than we did previously?

Kricheff: Yes.

Bogoch: How much smaller? The ones we saw were large, i.e., 5 cm in diameter.

Kricheff: It is difficult to give a precise answer because resolution in CAT scans depends on how much the absorption varies from surrounding areas. With a high difference in absorption, e.g., in an aneurysm with a lot of contrast material in it, lesions have been detected well below 1 cm; a metastatic tumor may get just as dense.

T. Hoshino: Dr. Cooper, have you seen any CAT scans that show the extent of a glioblastoma, even if polycentric, and thus help us to decide whether to use one big field rather than the whole brain?

Cooper: I do not think I can answer your question because I am not a radiotherapist. I can say that we obtain better resolution with the CAT scanner and have picked up multiple metastases that we do not observe with the radionuclide brain scan, because we have reached the limit of resolution for the small lesions less than 1 cm.

Ransohoff: The case material of a center is determined by the pool from which it draws. We have an active melanoma program in our hospital, so our intake of melanoma is higher than in other institutions. The number of patients with lung cancers submitted to craniotomy is much lower than the number of such carcinomas seen in the institution. We hesitate to operate on patients with carcinomas of the lung because they are notoriously bad tumors and frequently multiple. We often put these patients on steroids, give them radiation therapy, take them off steroids, and follow them. Some of the patients in the series I presented today were watched for 3 and sometimes 6 months before they were submitted to surgery. In short, each patient is different and I have no formula for determining who should be operated upon.

Surgical Management of Malignant Brain Tumors[1,2]

Joseph Ransohoff[3]

ABSTRACT—Data are presented to support the contention that aggressive surgical management of malignant brain tumors, both primary and metastatic, is valuable in terms of quality and length of survival. Metastatic brain tumors must be solitary in a patient whose primary is either asymptomatic or under good control to justify surgical removal. Primary malignant gliomas are surgical candidates when the core of tumor can be excised, thus increasing survival after radiation and chemotherapy.—Natl Cancer Inst Monogr 46: 145–150, 1977.

METASTATIC BRAIN TUMOR

In carefully selected patients, a total excision of solitary intracranial metastasis from a systemic malignancy can prolong life by many months. Continued advances in central nervous system (CNS) diagnosis verify the location of single tumors; the judicious use of corticosteroids in the preoperative and postoperative period and careful planning for surgical intervention can achieve low rates of postoperative mortality and morbidity. The selection of patients for metastatic brain tumor surgery requires input from several disciplines, including neurology, neuroradiology, internal medicine, oncology, and radiation therapy, if worthwhile results are to be obtained.

Patients with intracranial metastatic disease present in three distinct modes that must be considered separately. Those least likely to benefit from intracranial surgery come to medical attention with signs and symptoms of a systemic neoplasm; they may not have signs of CNS disease, and the intracranial tumor is usually uncovered during diagnostic evaluation. However, the symptomatic primary disease of the patient who has a silent though demonstrable intracranial metastasis rarely carries a prognosis that warrants consideration of intracranial surgery.

On the other hand, the patient admitted with CNS signs and symptoms, who is found to have a solitary intracranial tumor, may be in a different category if investigation demonstrates no primary source. The major basis for this difference in prognosis is the inadequate tumor defense mechanism in the CNS as compared with the systemic immunologic responses evoked by malignancy. In other words, these patients have contained their systemic disease, but a tumor embolus has escaped immunologic surveillance and is growing in the brain. These patients are the best candidates for intracranial surgery. Within the same group, but with a less favorable prognosis, are patients who have a symptomatic brain tumor and an asymptomatic, demonstrable primary on subsequent investigation.

Between these two extremes are patients who have been receiving treatment for primary systemic tumors for months or years before the appearance of CNS signs. This third group requires the most careful judgment. Once the physician has evidence that the patient probably has a single intracranial metastasis that can be excised with a reasonable operative mortality and morbidity, the deciding factor in the choice of therapy becomes the patient's response to prior treatment for the primary tumor and his prognosis independent of the CNS lesion. A patient with malignant melanoma often falls into this group. Between the treatment of the initial melanoma and the appearance of an intracranial tumor, he may have an interval of good health lasting as long as 8–10 years. Finally, patients with multiple intracranial tumors are almost never candidates for surgical intervention.

In an excellent review of the diagnosis and treatment of metastasis to the brain at Memorial Sloan-Kettering Cancer Center, Posner (*1*) described 171 intracerebral metastases discovered in a series of 1,905 autopsies in a 3-year period between 1970 and 1973. Even at autopsy from this cancer center, over one-third (65) of these metastatic lesions were single. In a series of

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga. February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Supported in part by Public Health Service contract N01 CM67010-18 with the Division of Cancer Treatment, National Cancer Institute, and by a grant from the Veterans Administration Brain Tumor Study Group.

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Table 1.—*Primary site of 100 surgically excised metastatic brain tumors*

Site	No.
Lung	28
Unknown	22
Breast	15
Melanoma	14
Genitourinary	10
Others	11

patients undergoing intracranial surgery for metastatic brain tumors, Lang and Slater (*2*), Storbecker (*3*), Vieth and Odom (*4*), Richards and McKissock (*5*), and Raskind et al. (*6*) reported a consistent distribution of the primary malignancy, and a review of 100 consecutive cases from our medical center (table 1) revealed a similar distribution. Carcinoma of the lung far outstrips all other tumors as a source of intracranial metastasis, accounting for up to 65% in some series. The second largest group in most surgical series are those who, as a group, have the best outlook. In them the primary cannot be determined before craniotomy, nor can a site be firmly assigned from pathologic study of the specimen removed at surgery and on additional postoperative search. In decreasing frequency thereafter, the remaining primary sites include breast, kidney, gastrointestinal tract, melanoma, and a host of rare tumors.

The neurologic signs and symptoms in patients with metastatic brain disease have no particular characteristics but are similar to those seen with most intracranial mass lesions, including the gliomas. A high percentage of patients with intracranial metastasis have headaches associated with personality change, memory loss, and mild confusion. These symptoms along with nausea and vomiting provide evidence of increased intracranial pressure and diffuse cerebral dysfunction, which are usually due to the extensive brain edema seen so often in association with metastatic brain tumor. The other more specific signs seen in patients with metastatic brain disease include seizures, either focal or generalized, hemiparesis, and findings such as aphasia, ataxia, and visual field defects. In contrast to patients harboring primary tumors of the CNS, either malignant or benign, patients presenting with dysfunction of the CNS due to metastatic brain disease may give a history of significant weight loss and malaise, which is unusual in primary disease of the CNS.

Similar diagnostic measures are used in the systematic evaluation of a patient with intracranial tumor irrespective of its nature. The minimal, general work-up should include posteroanterior and lateral chest films and tomography, if indicated, routine blood and urine determinations, and sedimentation rate. Of 158 patients with metastatic brain tumors described by Storbecker (*3*), only 25% had a sedimentation rate below 10 mm/hour; another 25% had a sedimentation rate between 10 and 20 mm; and the remaining 50% were over 20 mm. Except in those with a previous history of malignant disease, the presence of intracranial metastases is usually suspected on the basis of specialized neurologic studies. The application of corticosteroids to manage increased intracranial pressure has reduced the urgency of most diagnostic work-ups, and has allowed time for a carefully planned program of evaluation. If neurologic studies suggest a single metastatic brain tumor, a search usually can be conducted for the primary metastasis or for other metastases, at least to the extent of chest tomography, an intravenous pyelogram, mammography, and liver and bone scans. In view of the low incidence of gastrointestinal tract metastases to the CNS, preoperative studies of this system are rarely indicated. Such patients may also be subjected to nonspecific immunologic challenge with mumps vaccine and streptokinase–streptodornase (Varidase) (Soloway A: Personal communication); if they do not respond, they are probably in a state of anergy from carcinomatous disease and are poor candidates for operation.

The evaluation of a patient with signs and symptoms suggestive of an intracranial mass usually employs specific neurologic studies in a routine sequence, generally starting with radiographs of the skull, electroencephalogram, and brain scan. Skull films may show thinning and erosion of the dorsum sellae and posterior clinoids, and thus suggest increased intracranial pressure of long standing. Occasionally, a shift of a calcified pineal gland and evidence of local hypervascularity of the skull may indicate the site of a tumor, either a metastatic lesion or a meningioma, particularly if it is receiving a significant blood supply from the dura mater. The electroencephalogram is only of lateralizing value, although diffuse slowing is seen in association with intracranial pressure. The radioactive brain scan (^{99m}Tc) can be of considerable value, and operative treatment is excluded if there is clear-cut evidence of multiple lesions. It must be remembered, however, that brain scan does not demonstrate all metastatic tumors. The lower limit of tumor size demonstrated is approximately 0.5–1 cm, and 1 tumor may "light up" on brain scan and a second may not be seen at all or may show only as a faintly suspicious shadow. This phenomenon probably is due to the fact that in an individual patient, an intracranial metastasis may produce a great deal of edema in the surrounding white matter, whereas another tumor in the same patient in the opposite hemisphere may produce little or none; hence, a less visible pick-up on scan.

During the initial phase of evaluation, patients should be placed on anticonvulsant therapy (diphenylhydantoin, 0.1 g, three times daily) even if they have not had seizure phenomenon before admission, and corticosteroids should be added if they have signs of increased pressure or focal neurologic deficit. We prefer methylprednisolone, which generally is started at 120 mg/day and is maintained at this level throughout the diagnostic evaluation and up to the time a decision is made concerning surgery, radiation therapy, chemotherapy, or combinations thereof. Recent experience with massive doses of corticosteroids (*7*) (1–1.5 g methylprednisolone/day) has shown dramatic reversal of severe obtundation and marked neurologic deficit in patients with massive brain edema.

When it is available, computerized transaxial tomography (CTT or EMI) is of great value in the diagnostic work-up of patients with intracranial tumors. Ventricular size and position are seen clearly. Patients with a posterior fossa lesion (even those without signs of papilledema) will almost always show enlarged ventricles and a shift of the fourth ventricle. The iv injection of 200 cm^3 sodium diatrizoate (Hypaque) greatly enhances the definition of metastatic tumors and meningiomas, but primary gliomas do not "light up" as dramatically. Finally, bilateral cerebral angiography via a femoral catheter provides a definitive diagnosis except in those patients with a posterior fossa tumor in whom ventriculography may also be required on rare occasions. Lumbar puncture rarely is necessary in the work-up of a brain tumor patient unless one suspects intracranial infection or is searching for malignant cells in the cerebrospinal fluid.

Surgical excision of intracranial metastases should be limited to those patients in whom a thorough work-up appears to have demonstrated a single intracranial lesion. Deeply placed lesions, either primary or metastatic, in the mid-portion of the dominant hemisphere are not surgically accessible. However, most metastatic tumors, arising as they often do from the cortical gray–white junction, are accessible to the surgeon. Except when there is a strong possibility of a brain abscess, needle biopsy may be of considerable academic interest but, because of the risk, should be done only when it will provide information that will influence therapy significantly, e.g., the choice of a chemotherapeutic agent.

When the patient is submitted to operation based on the location of the lesion, the patient's age, his general medical status, the host–tumor relationship, and surrounding social factors, the surgeon sets out to see the patient through safely and to secure the best result possible. The operation must be planned carefully, with a wide exposure of the brain, preferably including one polar aspect, either frontal, temporal, or occipital. This will permit complete or nearly complete excision, and, if needed, an additional lobectomy can be done to provide adequate internal decompression. Fortunately, metastatic tumors commonly develop a pseudocapsule, and often the gross lesion can be excised intact. The bone flap is reseated firmly at the completion of the procedure. Rarely is there any justification for discarding the bone and leaving the dura open to provide an "external" decompression. This procedure, which generally indicates inadequate planning and poor execution, results in further herniation of viable brain and the development of a bulging skin flap; the patient may linger in a terminal state. The planned approach has little place for the intraoperative frozen section to determine the nature of the lesion because in most instances, the surgeon is committed to a definitive procedure that he decided upon before committing the patient to surgery.

Objective evaluation of the results of surgical extirpation of single metastatic disease is difficult. As Posner (*1*) pointed out, no one has done a random study comparing the effects of surgical extirpation and radiation therapy to those of radiation therapy alone. This type of study can be done only in patients in whom systemic tumor has been demonstrated. Advances in chemotherapy and immunotherapy probably will make such research more difficult to conduct in view of the varying responses of the CNS to these therapies. We believe all patients who have undergone a successful removal of an intracranial metastatic tumor should be given postoperative radiation therapy. The Radiation Therapy Oncology Group has been investigating various treatment regimens in a random study of the palliation of metastatic brain disease. After having cooperated in this study, Drs. J. Newell and R. J. Carella of the Department of Radiation Therapy at New York University recommend 4,000 rads in 10 treatments or 5,000 rads in 15.

TABLE 2.—*Results of combined surgical, radiation, and/or chemotherapy*

Tumor origin	No. of cases	Survival, mo					
		< 1[a]	1–6	6–12	12–18	18–24	Over 24
Lung[b]	28	5	11	3	4	2	3
Unknown[b]	22	2	3	8	5	2	2
Breast[b]	15	0	5	3	4	0	3
Melanoma[c]	14	2	2	3	1	2	4
Genitourinary[b]	10	1	3	4	2	0	0
Others	11	0	4	3	2	1	1
Totals	100	10	28	24	18	7	13

[a] Tumor was considered to be operative: mortality rate, 10%.

[b] All received radiation therapy.

[c] Patients with melanomas received various chemotherapies.

The effect of adding chemotherapy and immunotherapy to this regimen has not been evaluated adequately. When specific and well-established therapeutic regimens for the primary tumor are available, such as in melanoma, the patient should receive this treatment immediately and it should be continued for the duration of his life. The effects of some of the drugs currently under investigation for the treatment of primary brain tumors (glioblastoma), such as the lipid-soluble nitrosoureas and other promising agents, can be enhanced when they are administered in conjunction with radiation therapy. These drugs may be valuable in the treatment of metastatic brain disease, but once again, no authoritative random study has been conducted to determine their usefulness.

The literature contains many reports of surgical removal of metastatic brain tumor. Lang and Slater (*2*) reported a median survival time of 4 months and an average survival time of 12 months in 208 patients undergoing intracranial surgery for metastatic brain tumor. Richards and McKissock (*5*) described 108 patients in whom "total excision" of the tumor was achieved; 22 of them survived for 1 year or more. In a recent series from Memorial Hospital (*1*), those patients who received surgery and radiation therapy had a median survival time of 40 months and 21% survived for 1 year or more. Raskind et al. (*6*) reported 50 patients who underwent surgery for what appeared to be a single metastatic tumor; 15 of them survived for more than 1 year. Table 2 shows our results in the last 50 patients who underwent intracranial surgery for what appeared to be single metastatic tumors; they are similar to Posner's results.

Our experience suggests that selected patients with an apparently single intracranial metastatic tumor in a surgically accessible region have a 25% chance or better of meaningful survival for 1 year or longer following surgical removal and radiation therapy. The decision to operate on a patient who has had known malignancy before the onset of cerebral symptoms must be based not only on the CNS factors, but on the host's response to the primary tumor and results of therapy administered thereto. The patient whose systemic tumor presents itself primarily with CNS symptoms or signs may benefit significantly from intracranial surgery and radiation therapy. In the future, advances in chemotherapy and immunotherapy may further prolong life, particularly as progress is made in systemic and CNS tumor therapy. Thoughtful planning, multifactored decisionmaking, and aggressive surgery are essential for success in the treatment of metastatic brain tumor.

GLIOMA

The role of intracranial surgery in the treatment of malignant gliomas must be justified on the basis of good surgical principles as well as on those of sound cancer therapy. In and of itself, surgery never has and never will cure glioblastoma or malignant glioma grade III or IV. Furthermore, an intracranial procedure is not necessary for diagnosis because this often can be established from the history, the abnormal vascularity seen on cerebral angiography, and the appearance on radioactive brain scan and CTT scanning. If a definitive tissue diagnosis is desired, a needle biospy through a burr hole can be performed with minimal risk in this tumor.

An intracranial operation is justified to remove the great mass of the tumor, thus reducing the number of malignant cells to be exposed to radiation, chemotherapy, or immunotherapy. In malignant gliomas in which the rapidly growing invasive cells are at the margin of the tumor and the center is relatively avascular and at times necrotic, the removal of the central mass probably removes the area where viable cells persist and are hardest to reach with chemotherapeutic agents. Many investigators believe that these resistant cells account for the continued relentless regrowth of these tumors.

Successful intracranial surgery must meet the criteria of low mortality and of low morbidity and, at the same time, remove a large percentage of the tumor cells. To achieve these goals, careful planning must be done before intracranial surgery, as "wounding" or minimal removal of these vascular tumors often incites considerable swelling of the residual tumor and the surrounding brain. Routine preoperative evaluation includes brain scanning and cerebral angiography and, more recently, CTT scanning, the latter being extremely useful in the planning of radical glioma surgery. The CTT scan often demonstrates that the bulk of the tumor is restricted to the central white matter and displaces the ventricular system, whereas on brain scan and angiography, the same tumor would appear to be within structures such as the internal capsule and deep central gray masses which, if violated at the time of operation, would lead to irreversible paralyses and/or coma.

Radical removal can be performed in almost every intracranial location except the deep central areas of the dominant hemisphere, the posterior bilateral corpus callosum, and within the brain stem itself. Large doses of corticosteroids given before and after the operation, supplemented by intraoperative osmotic diuretics, will prevent disastrous brain swelling during the procedure and allow for the replacement of the bone flap. The bone flap should not be discarded in these patients any more than in those with metastatic tumor. Finally, operations should not be undertaken to confirm diagnoses but used to achieve the objectives outlined above.

The results of operations to remove the tumor mass performed on 30 consecutive patients with glioblastomas in the past 2 years afford some indication of the results that can be expected. Fourteen women and 16 men were in the group; the average age was 56 years with a spread from 21 to 73 years. This series contained 18 right-sided and 12 left-sided tumors, confirming that deep tumors in the center of the dominant hemisphere do not lend themselves to radical removal (table 3).

TABLE 3.—*Location of 30 grade IV gliomas*

Intracranial site	Location	
	Right	Left
Frontal	1	4
Frontotemporal	6	4
Tempoparietal	4	1
Parieto-occipital	2	2
Frontoparietal	2	1
Holohemispheric	3	0
Total	18	12

To evaluate the results of radical surgical removal, we rated these patients after operation according to the Karnofsky scale, which is based on the patient's ability to perform the functions of daily living (table 4). It is important to note that patients with occipital tumors could not achieve a rating above 80 because a postoperative hemianopic defect automatically placed them in that category even if they were normal in every other aspect. Table 5 shows the maximum rating achieved by these 30 patients 2 months after the operation; as can be seen, 19 of the 30 achieved a rating of 80 or better. In 1 patient who died during the first 2 months after surgery, the glioblastoma recurred dramatically despite radical removal, radiation therapy, and chemotherapy. Two patients failed to complete chemotherapy. In this table, the words "radical surgery plus" mean that during the postoperative period, all patients received radiation therapy and a number received chemotherapy, based on the Brain Tumor Study Group Protocol No. 72-01.

TABLE 4.—*An abbreviated Karnofsky rating*

Description	Scale, %
Normal	100
Normal—minor symptoms	90
Normal activity with effort	80
Self-care	70
Occasional assistance	60
Frequent care	50
Disabled	40
Severely disabled	30
Very sick	20
Moribund	10

TABLE 5.—*Maximum Karnofsky rating 2 mo after radical surgery, 1973-75*

Rating scale, %	No. of patients	No. surviving[a]
100	4	1
90	12	5
80	3	2
70	2	1
60	4	2
50	1	1
40	1	1

[a] Of the 30 patients, 19 had a rating of 80 or better and 27 completed surgery and postoperative therapy; 1 died during the first 2 months, and 2 failed to complete therapy.

TABLE 6.—*Maximum Karnofsky rating after radical surgery plus, 1971–73* [a]

No. of patients	Scale, %
1	100
6	90
3	80
6	70
3	60
1	50
5	40
0	30
1	20
1	10

[a] Ten of 27 patients had a rating of 80 or better.

The principal factor added to our diagnostic program during the 2 years of the study was the CTT scan, which can be assumed to have accounted, in part at least, for the difference in the Karnofsky ratings of the patients operated in the previous 2 years (table 6). Before the CTT scanner was available, only 27 patients, a little more than one-third, achieved a Karnofsky rating of 80 or better, whereas almost two-thirds achieved this rating in the subsequent 2 years.

Although this presentation does not establish the value of radical surgery in the treatment of malignant gliomas, we demonstrated that a significant proportion of patients can expect a good postoperative response after an energetic surgical attack on their tumors.

CONCLUSION

I have presented data to support the contention that aggressive surgical removal of malignant brain tumors, both primary and metastatic, can be performed with low mortality and morbidity rates in carefully selected patients. Operative removal thus appears to have a role in total treatment of these tumors because it improves quality and length of survival and increases the efficacy of other therapies directed toward the destruction of residual tumor cells.

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Corticosteroid Therapy in Patients With Brain Tumors[1]

Philip H. Gutin[2]

ABSTRACT—Since the first half of the last decade, numerous authors have testified to the clinical usefulness of corticosteroids in patients with brain tumors. The mechanism of benefit of these agents is controversial. Although it is clear that corticosteroid therapy reduces cerebral edema around brain tumors, mounting experimental evidence suggests that steroid compounds also may have a direct antineoplastic effect on brain tumor cells. The principal disadvantage of such therapy is that it makes difficult the evaluation of the tumor's response to oncolytic agents simultaneously administered. The most effective dosage of steroid varies; some patients require 96 mg dexamethasone daily before the maximum effect is achieved. These differences in response may be due to varying degrees of peritumoral edema, normal differences in steroid metabolism, and complex drug-to-drug interactions. Corticosteroid therapy may increase the incidence of gastrointestinal bleeding in neurosurgical patients, but this low risk is acceptable in view of its many advantages.—Natl Cancer Inst Monogr 46: 151–156, 1977.

The history of the early use of corticosteroids in patients with brain tumors is vague in the medical literature, but several papers are prominent. Ingraham et al. (*1*) used corticosteroids to ameliorate postoperative adrenal insufficiency in patients undergoing intracranial parasellar surgery but were most impressed by their effect on postoperative cerebral edema. Kofman and his co-workers (*2*) gave steroids to patients with primary and metastatic brain tumors in the hope that such hormone therapy would discourage tumor growth. To the surprise of these investigators, improvement was rapid and dramatic, and they concluded that it was due to an antiedema effect. Galicich and French (*3–6*) first used steroids specifically for this effect on cerebral edema; and in a series of papers spanning the first half of the last decade, they reported their results with a large series of patients with preoperative and postoperative brain tumors. This work has guaranteed steroid therapy its place in the neurosurgical armamentarium.

These early clinical successes prompted wide usage of corticosteroid agents for palliative control of cerebral edema in patients with primary and metastatic brain tumors; but those with metastatic disease received the most attention, perhaps because French (*6*) noted that this group responded most favorably to steroids. Ruderman and Hall (*7*), Horton et al. (*8*), Beresford (*9*), and Gottlieb et al. (*10*) also testified to the clinical usefulness of corticosteroids in patients with brain metastases. Primary intracranial tumors have received some attention in the large series that reported chiefly brain metastases. These patients also responded dramatically to corticosteroids (*11–15*).

Several investigators have quantitated the clinical responses to corticosteroids. Verdura et al. (*16*) described a patient with a brain metastasis from a bronchogenic carcinoma in whom the angiographic mass effect decreased markedly, concomitant with clinical improvement after prednisone therapy. Weinstein's group (*17*) described similar angiographic improvement with restoration of regional cerebral blood flow to areas near brain metastases when dexamethasone produced improvement in neurologic function. Kullberg and West (*18*) administered steroids to patients with brain tumors and measured reductions in ventricular fluid pressure waves that paralleled clinical improvement. Gärde (*19*) showed echoencephalographic reductions in midline shift when patients with brain tumors received steroids.

MECHANISMS OF ACTION

It is now generally accepted that corticosteroids are beneficial in the management of cerebral neoplastic disease, but the mechanism by which this benefit is produced remains controversial. Clearly, corticosteroids exert a profound influence on intracranial fluid balance, and this must at least partially account for the clinical improvement seen in brain tumor patients receiving steroid therapy. Equally important may be a direct antitumor effect on brain tumor cells. Each of these mechanisms will be discussed.

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Effects on Intracranial Fluid Balance

The brain contains three fluid compartments: the brain tissue water, the cerebrospinal fluid (CSF), and the brain blood volume (*20*). Corticosteroid therapy probably can influence each of these compartments to some degree and can ameliorate the disruptions in them caused by intracranial tumors.

Brain Tissue Water

Before considering the impact of corticosteroids on the disruption of brain tissue water, we should review the nature of the disruption itself, a process broadly referred to as cerebral edema. Klatzo (*21*) recognizes two forms of brain edema: The cytotoxic type, caused by direct cellular injury and seen in the white or gray matter, is characterized mainly by cellular (particularly astrocytic) swelling and little if any increase in cerebrovascular permeability. The vasogenic type is the result of increased vascular permeability and is seen (with varying degrees of the cytotoxic type) as the predominant form of cerebral edema accompanying brain tumors. Vasogenic edema is primarily a white matter process in which the disruption of the normal cerebrovascular barriers permits an egress of protein-containing edema fluid into the extracellular space. Experimental models of vasogenic edema show extravasation into the central nervous system (CNS) of markers that normally are excluded by the intact selective permeability of cerebral vessels (*22*). In addition to these morphologic changes in cerebral edema, distinct electrolyte and fluid shifts can be demonstrated in .the edematous brain tissue of patients and experimental animals. In such tissue, sodium and water are increased and potassium concentrations are decreased (*23, 24*).

Steroids have important effects on each of the morphologic and biochemical changes associated with the cerebral edema process. Long et al. (*25*) studied surgical specimens of edematous white and gray matter associated with brain tumors and, predictably, found electron microscopic changes that represented a combination of cytotoxic and vasogenic edema. They showed that dexamethasone in their patients favorably altered these histologic abnormalities to degrees that correlated with the clinical response to steroids. Experiments in animal models have demonstrated that the increased cerebrovascular permeability to normally excluded markers associated with vasogenic edema can be normalized by steroid therapy (*5, 26–29*). The water and electrolyte shifts described above can likewise be corrected in the edematous brain of experimental animals by steroid therapy (*30–33*).

Cerebrospinal Fluid

Whereas the major intracranial fluid alterations produced by steroids take place within the brain tissue water, the CSF and brain blood volume also are affected to some degree. These minor fluid shifts may contribute to the overall improvement seen with corticosteroid therapy in brain tumor patients.

Weiss and Nulsen (*34*) demonstrated significant decreases in CSF flow from implanted ventricular and cisternal drains when dexamethasone and methylprednisolone were administered, and Garcia-Bengochea found similarly decreased flows from the lumbar theca of cats given cortisone (*35*). Sato used ventriculocisternal perfusion techniques to demonstrate a 50% reduction in the formation of CSF in dogs treated with dexamethasone (*36*), yet this same technique in the monkey failed to show a convincing steroid effect (*37*). Whether corticosteroids do depress CSF formation and, if so, whether this has clinical significance remain to be proved.

Brain Blood Volume

Brain blood volume as reflected by cerebral blood flow is markedly reduced in the vicinity of brain tumors, and this flow impairment is reversed by dexamethasone therapy (*17, 38, 39*). The effect on brain blood volume is probably an indirect one, being principally mediated through the effects on brain tissue water. The reduction in cerebral edema may produce a decrease in tissue pressure and relieve compression and distortion of small cerebral vessels, and these changes would explain the measured normalization of the cerebral blood flow seen after steroids are administered (*38, 39*).

Antitumor Effect

Much less frequently considered than the antiedema effects of steroid compounds is their antineoplastic activity. Firm clinical data are scarce, but a good deal of experimental animal work suggests that corticosteroids do exert some antitumor effect. Gurcay et al. (*31, 32*) administered methylprednisolone to rats bearing intracerebral gliomas and found that survival was increased, cerebral edema decreased, and, most strikingly, dry tumor weights were reduced significantly when compared with controls. Shapiro and Posner (*33*) used dexamethasone in a similar study in mice with intracerebral ependymoblastomas. They confirmed the effects on survival, cerebral edema, and tumor weight, and also noted a significant reduction in the incorporation of tritiated thymidine into the DNA of steroid-treated tumors. Tumor-growth inhibition from steroids has also been observed in the intracerebral melanoma in mice (*40*) and in several glial tumors implanted sc in mice (*41, 42*) and rats (*42*). Wilson et al. (*43*) compared the in vivo proliferation kinetics of the rat glioma in methylprednisolone-treated and untreated animals and found that steroid treatment led to much longer doubling times.

Tissue culture data have confirmed that steroids inhibit the growth of cultured human glioblastoma (*44, 45*) and rat glioma (*43*) cells. Chen and Mealey, in their attempt to localize the site of steroid inhibition in the cell cycle, observed that methylprednisolone antagonized the mitotic arrest of vincristine in tissue culture and hypothesized that the steroid prevented the glial tumor cells from reaching the point of mitosis (*45*). No cytokinetic data that would localize the effect of corticosteroids upon cycling brain tumor cells have been found, although steroids probably inhibit the entry of leukemia lymphoblasts into DNA synthesis (*46*).

This experimental evidence is by no means conclusive. Geran et al. (*47*) tested a variety of steroid compounds against the intracerebral mouse ependymoblastoma screen and found no increases in survival. Furthermore, the extrapolation from animal data to the clinical situation appears hazardous. Claman (*48*) has observed that the sensitivity to corticosteroids (in the induction of lymphoid depletion) is extremely variable among animal species, man being relatively resistant when compared with the mouse. In the same spirit, Shapiro and Posner (*33*) warn that the steroid doses used in the mouse experiments outlined above are much higher on a milligram per kilogram basis than those given clinically. The importance of the antitumor effect of corticosteroids in animal brain tumors remains moot.

STEROIDS: A CONFUSING INFLUENCE

In recent years, the therapy of malignant cerebral tumors has grown more aggressive in response to the increasingly vigorous approach toward all malignant disease. However, progress in the treatment of brain tumors has been slow, in part because corticosteroids have been used so widely in patients under study. Measuring the response of a cerebral tumor to a chemotherapeutic agent is enormously frustrating because the current measures of response are vague and of variable accuracy (*49, 50*). The problem is compounded when steroids are given because the accuracy of the available measures is further compromised.

Corticosteroids influence and therefore obscure every known index of clinical response to brain tumor chemotherapy. Improvement in neurologic signs attributed to chemotherapeutic effectiveness could be due to the steroids often being given simultaneously. A decrease in the size of a brain scan tumor focus could be due either to steroid effect or to massive cell-kill after chemotherapy. Marty and Cain (*51*) and Fletcher et al. (*52*) demonstrated such scan improvement after dexamethasone in otherwise untreated patients who had abnormal brain scans from primary and metastatic tumors. Cerebral angiography, at best, provides a poor evaluation of chemotherapy because the correlation is weak between clinical and angiographic findings (*50*), and corticosteroids can reduce markedly the angiographic mass effect (*16, 17*). Even studies that measure only survival cannot escape the confusing influence of steroid therapy. Ruderman and Hall (*7*), for example, concluded that steroid therapy in their group of deteriorating patients with metastatic brain tumors stabilized the CNS disease to the extent that, in most instances, death was due to involvement of other systems.

Computerized axial tomography (CAT) offers some promise as a measure of chemotherapeutic effectiveness. Our pathologists and radiologists demonstrated a close correlation between density readings of this scan and pathologic material; this ability to differentiate tumor density from that of edema may enable us to separate the effects of oncolytic agents from those of corticosteroids. Our experience is yet too limited to permit firm judgments, but preliminary observations suggest that steroids may exert their greatest influence on the contrast enhancement of tumors, decreasing it considerably. Such decreased enhancement may represent a corticosteroid-induced stabilization of leaky vessels that prevents the egress of contrast material into the tumor bed. Antitumor agents seem to affect enhancement little while reducing tumor bulk, so this may prove a useful distinction. However, our optimism is carefully controlled as we gather further experience.

We must continue to look for indices of tumor response to oncolytic agents that are independent of the antiedema effects of corticosteroids. Radiologic procedures may not be the answer. The staff of the Brain Tumor Research Center of the University of California, San Francisco, is looking at in vitro cell viability of experimental tumors treated in vivo as a means for evaluating cell-kill (*53*). Taking a more molecular approach, our group is examining the damage done to the DNA of experimental tumors following in vivo therapy (Gutin PH, Hilton J, Fien VJ, et al: Manuscript in preparation). Perhaps both systems can be applied eventually to the clinical situation.

CLINICAL PRACTICALITIES

Dosage and Administration

Galicich and French (*3–6*) introduced dexamethasone (16α-methyl-9α-fluoro-prednisolone) into early clinical use for treatment of cerebral edema, selecting this drug for its known potency as an anti-inflammatory agent and its lack of mineralo-corticoid activity. Apparently, it was this early use that guaranteed dexamethasone its premier ranking among agents most frequently selected for the treatment of cerebral edema, although others have since proved equally effective. Recently, German workers have suggested that aldosterone is an effective antiedema agent, so the emphasis on low mineralocorticoid activity may not be an important clinical consideration (*54*).

Similarly, the early experience of Galicich and French established the commonly used dexamethasone dosage of 16 mg daily because in their studies this level appeared to provide the maximum clinical response. Dosages of this magnitude were used until recently when Renaudin et al. (*12*) showed that 1) against the edema associated with brain tumors, the steroid dose of maximum effect differed from patient to patient, and 2) some patients required up to 96 mg daily before they achieved an apparent maximum effect. Most investigators who deal with malignant brain tumors now go well beyond the 16 mg daily dose established by Galicich and French.

An optimal time schedule and route for steroid administration has not been defined. Corticosteroids are generally administered by the oral, iv, or im route every 4–6 hours, and this usually proves adequate. Experimental evidence suggests a precipitous drop in CSF cortisol levels 90 minutes after humans are given iv injections (*55*) and a similar time course in dogs (*56*). Hydrocortisone levels are undetectable in normal and edematous mouse brain 60 minutes after injection (*57*). This information appears to argue for more frequent administration of corticosteroids, especially when given iv, but current regimens by any route appear to be satisfactory.

After the administration of dexamethasone to patients with brain tumors, clinical improvement will appear in 8–48 hours (*3–6, 17*). Because of this slow onset of effect, osmotic diuresis (and other supportive measures) should be the choice in emergency situations.

Drug–Drug Interactions

The dexamethasone dosage required for maximum effect varied from patient to patient (*12*); although differences in tumor size and associated cerebral edema may explain this phenomenon, the known normal variation in dexamethasone metabolism may well play some part (*58*). This steroid metabolic pathway, already variable, is further influenced by complex drug–drug interactions (*59*). Diphenylhydantoin decreased the serum half-life of dexamethasone and increased the rate of appearance of its urinary metabolites (*58*), so this metabolic interaction may also partially account for the individual variations in the optimal dexamethasone dose in the large number of patients with brain tumors who received anticonvulsants. Phenobarbital, structurally related to diphenylhydantoin, also increases the excretion of the breakdown products of exogenously administered cortisol (*60*). Both anticonvulsants are potent stimulators of hepatic microsomal enzyme systems (*61*), and this mechanism offers an attractive explanation of the impact of these agents on steroid metabolism (*59, 60*).

Complications of Therapy

Every physician is familiar with the multitude of side effects associated with steroid therapy. The most potentially catastrophic complications of gastrointestinal (GI) ulceration and bleeding are of prime concern, and, consequently, most prescribe prophylactic antacids with steroids. French (*6*) reported GI ulceration and/or bleeding in only 4 of the 433 patients in his series. Maxwell and associates (*15*), in a recent update of their experience at the University of Minnesota, reported that most of the 4.7% of their patients who developed GI bleeding had only minor gastritis or esophagitis; serious hemorrhage was confined to those with severe head injuries and/or those in coma. Cantu et al. (*62*) concluded that steroid therapy increased fourfold the risk of serious GI hemorrhage in their comatose post-craniotomy patients.

It is the extremely ill, poorly compensated neurosurgical patient who is at high risk for GI bleeding. To what extent corticosteroids contribute to the propensity to stress ulceration in this group (*63*) is unclear. The specter of serious GI bleeding is always present in patients receiving steroid therapy, but the low incidence constitutes an acceptable risk in the management of disabling and potentially life-threatening cerebral edema.

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Needle Biopsy, High-Dose Corticosteroids, and Radiotherapy in the Treatment of Malignant Glial Tumors[1]

Lawrence F. Marshall[2] *and* **Thomas W. Langfitt**[3]

ABSTRACT—Biopsy and resection of a portion of the tumor have been valuable in the palliation of patients with malignant gliomas. However, in certain instances usually because of its location, resection of the tumor is impossible and, in these patients, the diagnosis can be established by needle biopsy. Twenty-nine patients with a needle-biopsy diagnosis of glioblastoma multiforme were submitted to a trial of combined radiation therapy and high-dose dexamethasone therapy. These patients received 6,000 rads of whole-brain radiation and thereafter were maintained on high doses of dexamethasone, i.e., 32 mg or more daily, until they succumbed. The patients were followed on a regular basis and the dosage of steroids was kept constant or adjusted upward according to the clinical examination. Seven of the 29 succumbed to their disease within 60 days after the biopsy. Of the remaining 22, 7 more died within 6 months. At the end of 1 year, 5 of the 29 were still alive and 3 had survived for longer than 18 months. Median survival was 29 weeks, and the mean survival was slightly longer, 34 weeks. In patients in whom surgical decompression is impossible, high-dose corticosteroid therapy will often yield an adequate chemical decompression. High-dose corticosteroid therapy used in combination with radiotherapy yields survivals comparable to those obtained with palliative resection and whole-brain radiation. As new chemotherapeutic combinations become available for malignant glial tumors, we believe chemical decompression will become increasingly important.—Natl Cancer Inst Monogr 46: 157–160, 1977.

A previous report discussed needle biopsy as an alternative to achieve a tissue diagnosis in patients with malignant glial tumors (*1*). This technique, which has proved safe and reliable, can be performed under local anesthesia. We reserve needle biopsy for patients with lesions in locations where, in the judgment of the surgeon, significant resection of the tumor is impossible because it is located in the deep gray matter, or because there is extensive involvement of the dominant hemisphere, often with extension across the corpus callosum. The present series excludes those patients in whom significant reduction of tumor bulk was possible, i.e., those in whom craniotomies were performed. Our experience over the past few years suggests that approximately one-third of the patients who present with supratentorial malignant glial tumors have neoplasms that defy significant bulk removal. These tumors create a dilemma for the surgeon; Jelsma and Bucy (*2*) in their classic paper on glioblastoma multiforme demonstrated that patients in whom bulk resection was impossible fared poorly. Because such patients deserve an attempt at therapy, we studied the influence of needle biopsy, high-dose corticosteroids, and radiation in them. Furthermore, we believed it was essential to have a tissue diagnosis in patients with "inoperable" lesions to assess the value of high-dose corticosteroid and radiation therapy.

MATERIALS AND METHODS

From September 1972 until July 1974, we admitted to the trial 29 patients from the Philadelphia Veterans Administration Hospital (now known as the Veterans Administration Health Care Center) and the Hospital of the University of Pennsylvania. The only criterion for entry was a permanent section diagnosis of glioblastoma obtained through needle biopsy. Patients were not excluded because of significant neurologic deficit or advanced age. Of these 29 patients, 18 (62%) were men and 11 (38%) were women. The youngest patient was 46 years of age and the oldest was 82 ($\bar{x}$=58 yr).

Immediately before the biopsy, all patients were started on dexamethasone in doses of 40 mg or greater daily; ($\bar{x}$=68 mg), given in 4 divided doses with an antacid. After needle biopsy, which was done under local anesthesia in 26 of 29 patients in whom a positive diagnosis was established, the patients were referred for radiotherapy with the objective of 6,000 rads of whole-brain radiation. Radiation was discontinued only if the patients developed progressive neurologic deficit that did not respond to an increase in dexamethasone or the administration of oral glycerol. In such patients the clinical course was one of steady deterioration. Those patients who completed radiation therapy were continued on high-dose dexamethasone; the lowest dose administered in this series was 32 mg daily. We adjusted the dose in terms of the neurologic examination. We undertook to evaluate the quality and length of survival, although the former was difficult because in most patients, the initial neurologic deficit was severe.

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Table 1 outlines the clinical profile in these 29 patients. Twenty-three were alert, 5 were drowsy, and 1 was deeply stuporous. Fourteen patients had severe dysphasia and 9 of them were also hemiparetic. Ten patients had significant changes in mentation and the remaining 5 were neurologically intact. These last 5 came under investigation because of personality changes or seizures. Three of these had bifrontal glioblastomas that crossed the corpus callosum and in 2 the lesion arose in the right thalamus. Thirteen of the 29 patients had papilledema at the time of admission. Table 2 indicates the location of the tumor.

RESULTS

No surgical fatalities were encountered. One patient with a deep thalamic lesion developed dysarthria and left arm weakness that gradually improved. The condition of 4 of the 29 deteriorated during radiotherapy and they did not complete the course; each died within 60 days of biopsy. Table 3 illustrates the survival data for the 29 patients. Median and mean survival for the group was 29 and 34 weeks, respectively. Seven patients died within 60 days of diagnosis. By 6 months 14 of the 29 had died, and at 1 year only 5 survived (17%); none survived longer than 20 months. All the long-term survivors (1 year or more) had lesions either in the deep gray matter, thalamus or basal ganglia, or in the corpus callosum.

The quality of survival was difficult to judge because many of the patients were severely impaired before biopsy. As others have observed, the initiation of corticosteroid therapy often has a dramatic effect on the clinical course, and patients could be kept from deteriorating further for significant periods by adjusting the steroid dose upward.

Of the 22 patients who survived more than 60 days, 13 showed a definite clinical improvement as the dexamethasone dose was increased. Attempts to reduce the dose (once the maximum of 80 mg/day had been reached) usually caused rapid deterioration. Five patients who eventually received 80 mg/day could tolerate no reduction of the dexamethasone, and 3 were maintained at this dose level for longer than 90 days. Dosage levels greater than 80 mg/day did not reverse new symptoms in any patient already receiving this maximum amount. Of the 14 patients who presented with dysphasia, the speech of 5 improved significantly during most of their survival. In 6, speech improved transiently but then deteriorated. In the last 3, steroids improved neurologic function only minimally, and all died before they completed radiotherapy. In the 9 hemiparetic patients, motor function improved significantly in all but 2; 5 of these were able to use the paretic side well until shortly before they died.

The 5 patients who were relatively normal neurologically at the time of tissue diagnosis remained in good health for a considerable period. All completed the radiotherapy and their mean survival was 432 days, which was considerably longer than that of the group as a whole (mean 236 days; $P < 0.05$). For these patients at least, high-dose corticosteroids and radiotherapy achieved significant and quality palliation.

TABLE 1.—*Clinical profile in 29 patients with glioblastoma multiforme*

Patient status	No.	Percent of total
Level of consciousness		
Alert	23	79
Drowsy	5	17
Stuporous	1	4
Neurologic		
Dysphasia	14	49
Mental changes	10	34
Hemiparesis	9	31
Intact	5	17

TABLE 2.—*Site of neoplasm in 29 patients with glioblastoma multiforme*

Site of involvement	No. of patients
More than one lobe dominant hemisphere	14
Thalamus	5
Central involving both hemispheres	5
Basal ganglia	3
Right hemisphere (ages 71 and 77 yr)	2

TABLE 3.—*Postoperative survival time in 29 patients* [a]

Length of time	No. of patients	Percentage
2 mo	22	76
6 mo	15	52
1 yr	5	17
2 yr	0	—

[a] Median survival=29 wk; mean survival=34 wk.

In the present series, few complications were encountered from the use of high-dose dexamethasone therapy. Three patients developed hyperglycemia that required reduction of steroid dosage and the administration of insulin. Five patients became severely cushingoid. Finally, 1 patient died with a severe electrolyte disturbance that may have been due to the high doses of dexamethasone.

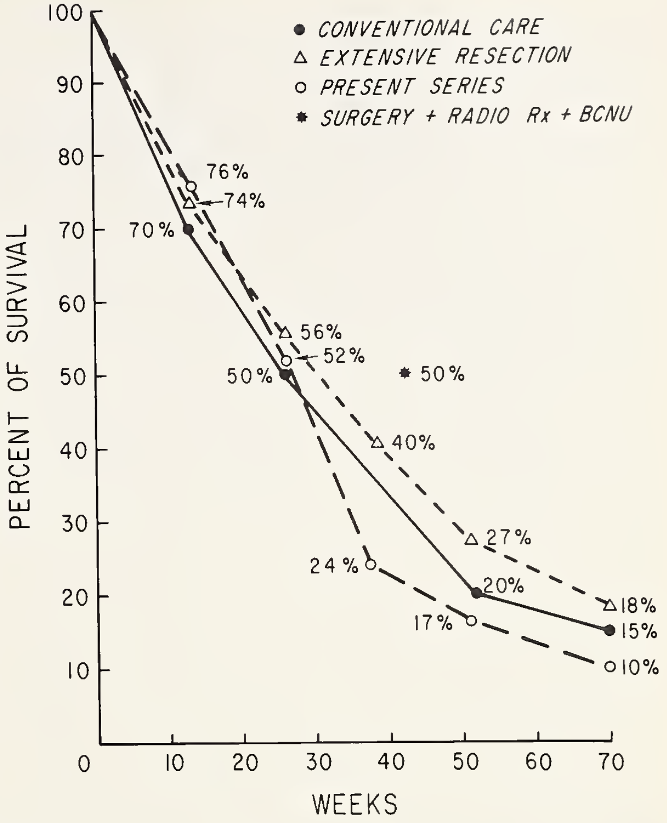
DISCUSSION

High-dose corticosteroid therapy in combination with radiation can produce significant palliation in some patients with glioblastoma multiforme. In vitro data have suggested that corticosteroids may have a specific tumoricidal effect in addition to their influence in controlling edema (*3*, *4*). Also, evidence from in vivo studies in leukemia and other myeloproliferative disorders indicate that corticosteroids may produce actual cell lysis (*5*). For these reasons, it can be speculated that dexamethasone in high doses may have a specific tumoricidal effect in glioblastoma.

In our experience, patients who failed to respond initially to high-dose corticosteroid therapy had an ominous course, which indicated that the biologic progression in these tumors was uncontrollable. These patients did not respond to radiation therapy. Of the 7 who died within 60 days, 6 showed minimal or only a transient response to high-dose dexamethasone.

The slope of the present survival data shown in text figure 1 resembles that obtained in other studies (*2*, *6*). At 3 and 6 months following diagnosis, the data points are nearly identical, whether the patient underwent extensive resection with radiotherapy or received high-dose dexamethasone and radiotherapy. Only Walker's patients (*6*) treated with surgery, radiotherapy, and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) showed significantly more survivals at 40 weeks (even so, 50% of his patients had already succumbed at this point).

These experiences suggest that any group of patients with glioblastoma multiforme includes a certain number with neoplasms of such biologic nature that no mode of available therapy can influence their clinical course. Thus to assess the efficacy of any therapy, we must look at patients whose clinical course is not one of inexorable deterioration from the moment of diagnosis. This assumption suggests that reduction of tumor bulk is important in the treatment of glioblastoma multiforme. Jelsma and Bucy (*2*) showed that of the patients in whom extensive resection and radiation therapy was possible, 10% were still alive 2 years after resection; at the end of 5 years, 5% of the entire group were still alive. Walker's (*6*) more recent series treated with surgery, radiation therapy, and BCNU also supports the view that tumor bulk reduction combined with radiation therapy helps a few patients. Nevertheless, the differences in survival between the various series are surprisingly small. The increased human and economic costs of craniotomy should persuade the neurosurgeon to reserve craniotomy for younger patients.



TEXT-FIGURE 1.—Survival of patients with malignant gliomas, 6 of whom were treated by conventional care; 1 by extensive resection; 6 by surgery, radiation therapy, and BCNU; and needle biopsy and corticosteroid therapy (present series).

Our data and patient population, unless they are much different from those of other neurosurgeons, suggest that we will continue to encounter considerable numbers of patients with glial tumors that defy even partial resection.

On the other hand, we should be encouraged by the fact that patients with little neurologic deficit but with deep lesions achieved significant palliation from high-dose corticosteroids and radiotherapy because these lesions are never likely to be operable.

We have concluded that high-dose dexamethasone therapy in conjunction with radiation will give significant palliation in patients with glioblastoma multiforme. This form of therapy is recommended only for patients in whom extensive bulk resection is contraindicated or impossible. If, in the future, better combination chemotherapies are developed for glioblastoma multiforme, chemical decompression with steroids may become even more important, particularly while chemotherapy is being initiated. It is also imperative that the use of corticosteroids be controlled strictly in clinical trials for testing chemotherapeutic agents. If such use is not controlled, the present study suggests that the interpretation of such trials will be extremely difficult and that they will have limited value.

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Chemotherapy Studies of Various Nitrosoureas: Prevention of Development of Ethylnitrosourea-Induced Tumors in the Nervous System[1,2]

Pietro Paoletti,[3] Maria T. Giordana,[4] Stefano Pezzotta,[5] *and* Davide Schiffer[4,6]

ABSTRACT—Of four nitrosoureas administered to F344 rats that were offspring of mothers treated with ethylnitrosourea during pregnancy, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea and its methyl derivative were particularly effective in blocking tumor development.—Natl Cancer Inst Monogr 46: 000–000, 1977.

The chemotherapeutic effect of the derivatives of the nitrosoureas on human and experimental cerebral tumors is already known (*1–5*). We set out to determine whether chemotherapeutic agents administered before the tumor developed were effective in prevention. The F344 rats used were offspring of mothers treated with ethylnitrosourea during pregnancy and thus made subject to tumor development. The agents were administered at regular intervals beginning 2 months after birth, as reported elsewhere (*6, 7*). Table 1 shows that (in the order of their efficacy) 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea(Me-CCNU), and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) markedly reduce the number of tumors that develop. The efficacy of 3-(tetra-acetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea (GCNU) is much less evident.

Given the number of rats available for every observation period, it is clear that these drugs delay tumor development. The preventive effect is appreciated by comparing the delaying action and the average life-span of the rat. Histologic study of tumors that developed despite treatment shows that morphologic modifications appear; we noted a prevalence of astrocytic gliomas and an absence of neurinomas after treatment with Me-CCNU. These data and their interpretation are reported by Schiffer et al. (*7*).

We have concluded that this experimental model is useful in the study of antitumoral chemotherapeutics for the nervous system.

TABIE 1.—*Effects of BCNU, CCNU, Me-CCNU, and GCNU on tumor development*

Age at administration, mo	No. of rats					No. of rats with tumor				
	Controls	BCNU	CCNU	Me-CCNU	GCNU	Controls	BCNU	CCNU	Me-CCNU	GCNU
6	6	4	12	15	8	4	0	1	1	0
9	15	29	20	2	10	11	8	1	1	8
14	7	9	6	—	3	4	2	1	—	1
Total	28	42	38	17	21	19	10	3	2	9
Percentage						68	24	8	12	43

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Hyperbaric Oxygen and Radiation Therapy in the Management of Glioblastoma[1, 2]

Chu H. Chang[3, 4]

ABSTRACT—A pilot clinical trial on radiotherapy of glioblastoma with and without hyperbaric oxygen was performed at the Columbia-Presbyterian Medical Center. Eighty previously untreated patients with histologically proved glioblastoma were evaluated; 38 were irradiated under hyperbaric oxygen and 42 (controls) in atmospheric air. The survival rates were calculated according to the actuarial analysis method. At the end of 18 months, the survival rate appeared considerably higher in the oxygen group (28%) than in the controls (10%). At the end of 36 months, no patients in the control group survived, whereas 2 patients in the oxygen group were alive beyond 45 and 48 months, respectively. The median survival time was 38 weeks for those treated under oxygen and 31 weeks for the air control group. Owing to the small population samples and the pilot nature of this study, the difference in survival rates between the two groups was not statistically significant. The toxicity of hyperbaric oxygen was well tolerated by most patients, and the quality of survival in the hyperbaric oxygen group was equal to or slightly better than that of the control group. This pilot clinical study paved the way for further controlled clinical trials of hyperbaric oxygen and oxygen-mimicking drugs, including the electron-affinic compounds that could have differentially sensitized the hypoxic tumor cells.—Natl Cancer Inst Monogr 46: 163–169, 1977.

Molecular oxygen has long been recognized as one of the most powerful modifiers of cellular radiation sensitivity. In 1953, Gray et al. (*1*) reported that the biologic effect of X-rays is increased by a factor of about 3 when X-irradiation is performed under well-oxygenated conditions as compared with anoxic conditions. The survival curves for mammalian cells exposed to X-rays in the presence and absence of oxygen are illustrated in text-figure 1. The shape of the survival curve is the same under aerated and hypoxic conditions. The only difference is the magnitude of the dose required to produce a given degree of biologic damage. At all levels of survival, the dose required under hypoxic conditions is about three times greater than that required under aerated conditions. This dose ratio between the hypoxic and aerated conditions is called the oxygen enhancement ratio (OER), which appears to be one of the most important parameters for the assessment of biologic effect of other radiation sources, such as radiation of high linear energy transfer, and of other radiation sensitizers such as hypoxic cell sensitizers.

Although the oxygen effect has been confirmed subsequently in many mammalian tissue culture systems and in experimental solid tumor models, clinical trials of radiotherapy of human cancer with hyperbaric oxygen have met with only limited success.

Malignant glioma is considered a unique tumor for use in a clinical trial of the effect of oxygen in radiotherapy because 1) malignant glioma runs a rapid and uniformly fatal course. Survival following neurosurgical decompression and conventional radiotherapy is greater than 90% mortality within 18 months, so that the treatment results can be assessed in a reasonably short period. 2) A high incidence of necrosis and hemorrhage is present in malignant glioma, which indicates that an anoxic or hypoxic tumor cell subpopulation exists. 3) Malignant glioma is confined to the cranial cavity, and an extracranial metastasis is exceedingly rare; the tumor can be completely encompassed in the irradiated volume without escape.

The available methods for restoring tissue oxygen tension in human tumors during radiotherapy include: 1) breathing pure oxygen under increased atmospheric pressure in a compression chamber (*2*); 2) breathing oxygen and carbon dioxide (95 and 5%, respectively) under atmospheric pressure through a nose mask (*3, 4*); and 3) intra-arterial injection of hydrogen peroxide in the tumor region (*5*).

Apparently, hyperbaric oxygenation is highly efficient because after breathing oxygen under

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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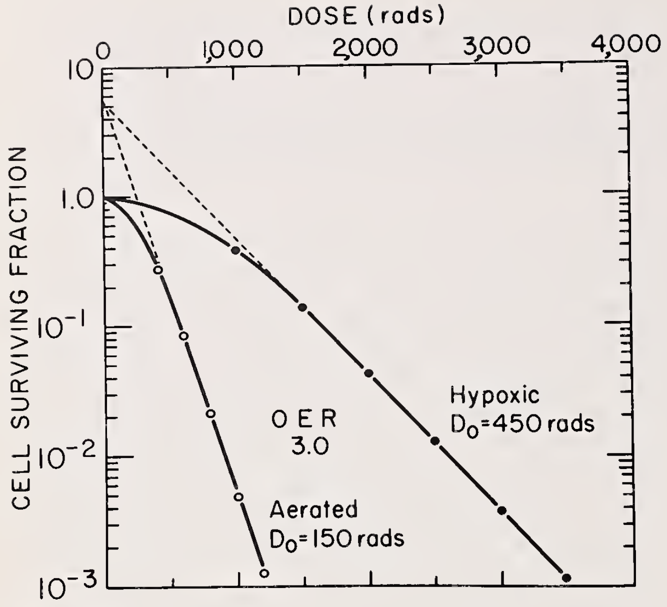


TEXT-FIGURE 1.—Survival curves for cultured mammalian cells exposed to X-rays under aerated conditions and under hypoxic conditions produced by passing a stream of purified nitrogen over the cells. These data are typical of many in the literature in which oxygen appears to be dose modifying. (Reproduced from Hall FJ: Radiobiology for the Radiologist, with the permission of the author and Harper & Row.)

high pressure at 3–4 atmospheres [(atm); (absolute)], the partial pressure of oxygen in the arterial blood and its solubility in physiologic solution may increase 20- to 30-fold, respectively (*6*). However, the response of cerebral blood flow to high pressure oxygen (HPO) remains a subject of controversy. Cerebral vasoconstriction was demonstrated in conscious man breathing oxygen at 1 atm by Kety and Schmidt (*7*), and at 3.5 atm by Lambertsen et al. (*8*). One could argue that vasoconstriction might negate the effort of forcing more oxygen to the brain and to the brain tumor under high atmospheric pressure.

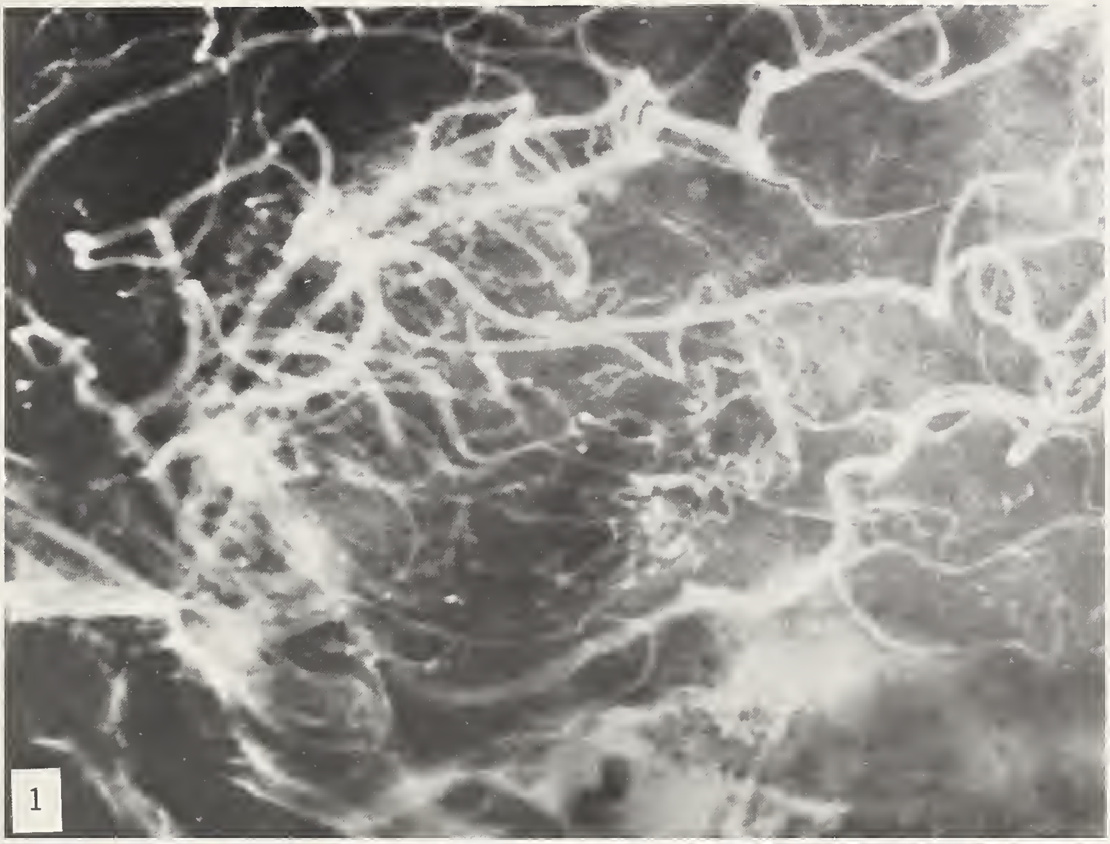
Recently, Ledingham et al. (*9*), using a radioactive Krypton clearance technique, demonstrated that anesthetized dogs exposed to pure oxygen under constant alveolar and arterial pCO_2 showed an 11% decrease in cerebral cortical blood flow to oxygen breathing at 1 atm, and a 21% decrease to oxygen intake at 2 atm. However at 3 atm, they found that oxygen stimulates an actual increase in cerebral cortical blood flow initially, with a later fall toward the normal air values. At no stage were they able to demonstrate a vasoconstrictive action with oxygen breathing at 3 atm. They attributed this lack of vasoconstriction at a higher pressure than 2 atm to cerebral oxygen toxicity, which manifests itself at 3 atm. However, Lambertsen et al. (*8*) attributed the cerebral vasoconstriction which, in their experiment, occurred in conscious man during oxygen breathing at 3.5 atm to arterial hypocapnia (decreased arterial pCO_2) resulting from associated hyperventilation, rather than to the direct effect of hyperbaric oxygen (increased arterial pO_2).

More recently, using a magnification cerebral angiographic technique, Wende et al. (*10*) reported a phenomenon in which an increased regional cerebral blood flow in the tumor region was obtained under hypocapnia and hypertension [the patient is hyperventilated for 10 min and this is followed by injection of the hypertensive drug Akrinor (Chemie Werk, Hamburg, Fed. Republic of Germany)]. Normal cerebral vessels reacted with vasoconstriction to a lower arterial pCO_2 value, whereas tumor vessels failed to show autoregulation. Under this circumstance, the tumor vessels receive more blood as the normal cerebral vessels constrict. The extent of the tumor can thus be shown more distinctly (figs. 1, 2).

In 1963, we acquired a new model of a transparent pressure chamber made of Lucite (Vickers Ltd., London, England). The chamber is operated at a pressure of 3 instead of 4 atm (absolute). At this lower pressure, the frequency of convulsions due to oxygen toxicity is greatly reduced and the need for general anesthesia is eliminated. With the elimination of general anesthesia, many more fractionated treatments can be given to the patients than on the previous schedule, which consists of only 2 or 3 fractions of massive doses (*11*). However, with the increase of the number of fractions, it is necessary to reassess the time–dose relationship and biologic response.

FIGURE 1.—Temporoparietal glioblastoma in a 69-year-old patient. Magnification cerebral angiography under normoventilation and normotension. Distinct contrast filling of the tumor vessels. [Reproduced with permission of S. Wende and Springer-Verlag (*10*).]

FIGURE 2.—Same patient. Magnification angiography under hyperventilation (hypocapnia) and hypertension. Constriction of normal cerebral vessels present. *Note* the tumor vessels are enlarged and more distinctly filled with contrast medium. A contrast enrichment of the early pathologic veins is also shown. [Reproduced with permission of S. Wende and Springer-Verlag (*10*).]



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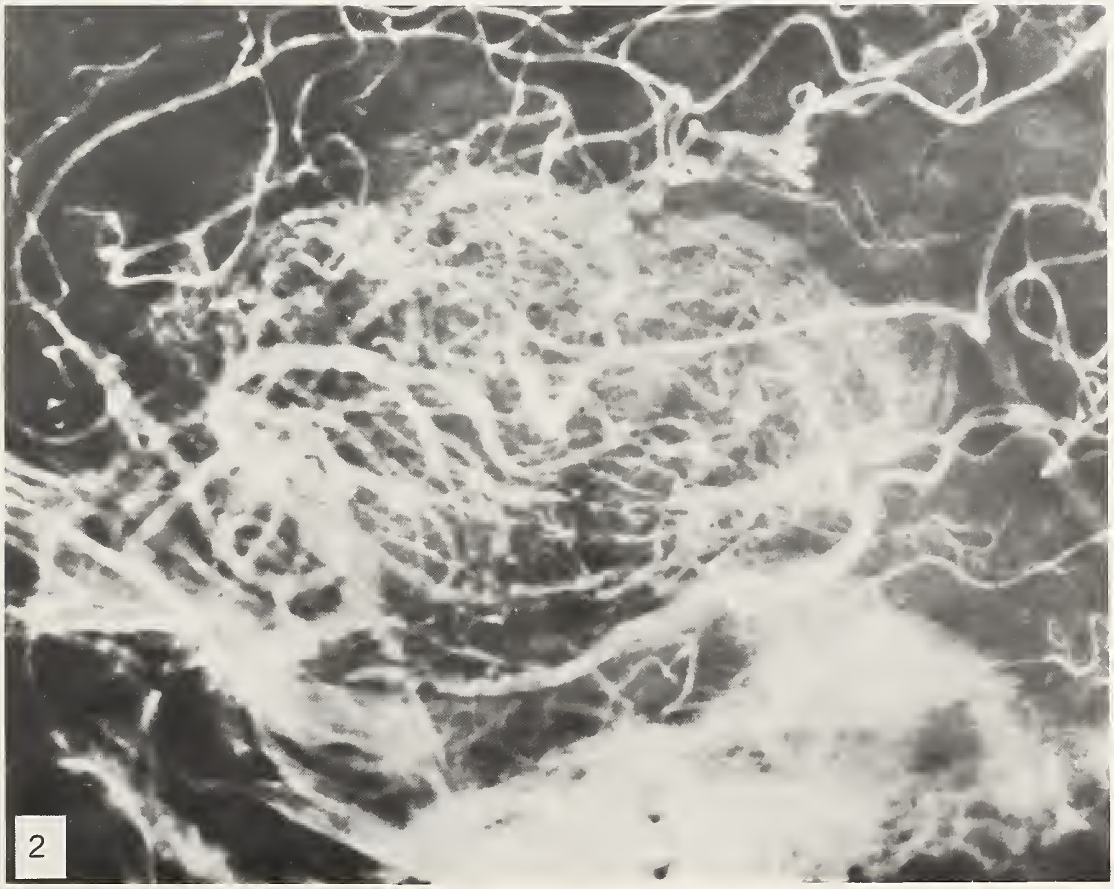

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MATERIALS AND METHODS

At the Neurological Institute of Columbia-Presbyterian Medical Center, New York, about 30 patients with malignant glioma are seen and treated each year, among which 15 have histologically proved glioblastoma (astrocytoma, grade III or IV). We began a pilot study (*12*) in late 1963, with an initial dose schedule of 3,600 rads in 3 weeks, i.e., 400 rads/treatment, 3 treatments/week (TIW). We used a radiocobalt source and a bilateral opposing wide-field technique. This was a conservative initial dosage, but we wanted to avoid the potentially deleterious effect of combined oxygen and radiation in unconventional fractionation. The dose was systematically increased to 4,000 rads in $3^1/_3$ weeks (400 rads, TIW); 4,400 rads in $3^2/_3$ weeks (400 rads, TIW), and 5,000 rads in 5 weeks (333 rads, TIW), as our experience with the oxygen toxicity and brain tissue tolerance increased. Since 1969, the dosage and fractionation have been changed to 6,000 rads in 6 weeks on a schedule of 5 treatments per week. The initial 3,000 rads are delivered to the whole brain under conventional aerated conditions, and only the final 3,000 rads are delivered under hyperbaric oxygenation to a reduced field size encompassing the demonstrable tumor volume, in keeping with the new knowledge that partial reoxygenation takes place in tumors during conventional radiotherapy with daily fractionation (*13*). The pilot study was designed to include eventually a pair-arrangement technique, whereby one in the pair was treated with pure oxygen breathing at 3 atm and the other under aerated conditions as a control. This technique was followed as closely as possible but was not in strictly random fashion because a difficult clinical situation and a complicated step-up change of dose fractionation schemes occurred.

Patients.—Eighty-one previously untreated patients with supratentorial glioblastoma were admitted to the project; 39 were treated with hyperbaric oxygen and 42 under normal atmospheric air as controls. One patient in the hyperbaric oxygen group died during the second week of treatment. Therefore, only 38 patients in the oxygen group and the controls were evaluated. The case distribution and fractionation scheme of the 2 groups are shown in table 1, whereas the age, sex, and tumor grade distribution in the 2 groups are compared in table 2.

TABLE 1.—*Number of patients at various dose levels in oxygen and control groups*

Dose range[a]	Group	
	Oxygen[b]	Control[c]
3,600 rads/3 wk or 1,540 ret	8	10
4,000 rads/$3^1/_2$ wk or 1,650 ret	4	4
4,400 rads/$3^2/_3$ wk or 1,750 ret	2	5
5,000 rads/5 wk or 1,780 ret	6	8
6,000 rads/6–7 wk (200 rads daily)[d] or 1,770 ret	18	15
Total	38	42

[a] *See* text for details; ret=rad equivalent therapy.

[b] HPO was given at 3 atm.

[c] Air was administered at 1 atm.

[d] Initial 3,000 rads were given in air for both groups; only last 3,000 rads were given under hyperbaric oxygen or in air by randomization.

TABLE 2.—*Case distribution of age, sex, and tumor grade in oxygen study and air control groups*

Parameters	Groups	
	Oxygen	Control
Age, yr		
Median	49	54
Range	23–68	33–77
Sex		
Male	24	27
Female	14	15
Grade of astrocytoma		
III	9	11
IV	29	31

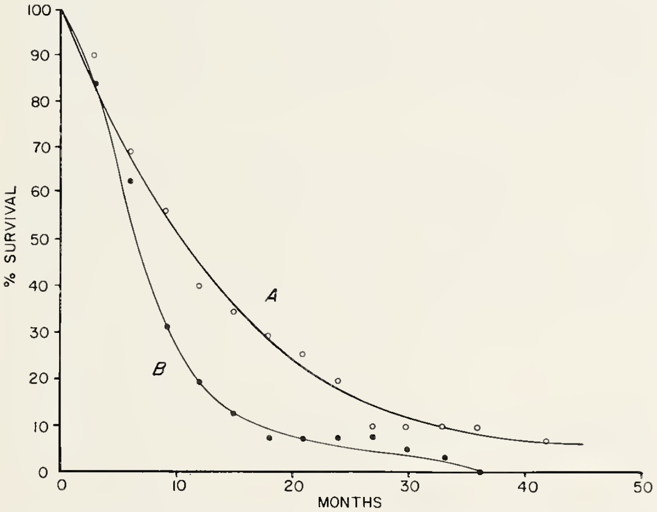
Treatment procedure.—The treatment is usually begun 1 day after the surgical sutures are removed from the scalp. Bilateral myringotomies are usually done 1 day before the course of radiotherapy. Premedication with a sedative (secobarbital sodium, 100 mg) was given im 30 minutes before the patient entered the pressure chamber. While the head was immobilized with a special tape holder, the localization port film was obtained. The pressure chamber was then flushed at atmospheric pressure with 100% oxygen until the oxygen content in the chamber reached 90%. The pressurization (which usually takes 5–6 min) was begun until a pressure of 30 pounds per square inch was attained (3 atm absolute). At this time, the oxygen concentration in the chamber is measured and is usually at 99+%, as indicated by a Beckman model D oxygen analyzer (Beckman Instruments, Inc., Fullerton, Calif.). The pressure was maintained at this level for 15 minutes for adequate oxygen diffusion from the cerebral capillaries into the tumor tissue before the irradiation was given. The decompression was started as soon as irradiation ended and took about 3–4 minutes. The control group was irradiated (without the pressure chamber) in normal atmospheric air but with the same dose and fractionation.

RESULTS

The results were evaluated in terms of survival rate, quality of survival, and complications. The survival period was calculated from the date of commencement of radiotherapy, i.e., the date after randomization, to the date of death.

Survival Rate

We applied an actuarial method of analysis, and the survival rate was depicted in two curves (text-fig. 2). Curve A (the survival rate of the oxygen group) is a slightly higher rate at every 3-month interval than the control group (curve B). At the end of 18 months, the survival rate appeared considerably higher in the oxygen group (about 28%) than in the controls (about 10%); at 24 months, the two curves tend to converge. However at the end of 36 months, the remaining 2 controls died, and 3 patients in the oxygen group were still alive. By the end of 40 months, 1 patient in the oxygen group died of tumor recurrence and the other 2 were still alive at 45 and 48 months, respectively, at the time this Symposium was convened. The median survival time in this series was 38 weeks for 38 patients in the oxygen group and 31 weeks for the 42 controls. Because of small population samples and the pilot nature of the study, no significant statistical difference in survival rates could be drawn between the 2 groups. The median survival time was 46 weeks for those 18 patients who received 6,000 rads in 6 weeks under hyperbaric oxygen and 25 weeks for the 15 who received 6,000 rads in 6 weeks as controls.



TEXT-FIGURE 2.—Glioblastoma-postradiotherapy survival rate. *Curve A* represents the study group (surgery plus radiotherapy plus hyperbaric oxygen) of 38 primarily treated cases. *Curve B* represents the control group (surgery plus radiotherapy only) of 42 primarily treated cases. Difference in survival rate between the 2 groups is most apparent at 15–18 mo after therapy. At the end of 36 mo, all patients in the control group had died, whereas 2 patients in the oxygen group were still alive at 45 and 48 mo, respectively.

The cumulative survival rates for the 38 patients in the oxygen group were 66% (25/38) at 6 months, 37% (14/38) at 1, 13% (5/38) at 2, 8% (3/38) at 3, and 5% (2/38) at 4 years, respectively, and for the 42 controls were 62% (26/42) at 6 months, 31% (13/42) at 1, 7% (3/42) at 2, and 0% (0/42) at 3 years, respectively.

Quality of Survival

About 25% of the patients who received radiotherapy under hyperbaric oxygen enjoyed a better quality of survival than the control group. For example, 1 patient who managed a supermarket returned to his job in full capacity for at least 6 months after a course of radiotherapy under hyperbaric oxygen. Another patient who was a banker returned to work for 1 year before he was advised to retire.

Complications

Most patients tolerated the hyperbaric oxygen procedure well. Some continued their therapy as outpatients after having received initial treatments for 1 or 2 weeks in the hospital. Only 2 patients developed a generalized convulsion at the time of decompression due to oxygen toxicity; the incidence is less than 0.5% in terms of the total number of exposures in our series. Several patients had mild otitis media after myringotomy and hyperbaric therapy. One patient developed a severe skin reaction with subsequent disruption of the scalp wound. Three patients had extensive radiation necrosis of the adjacent normal brain tissues observed at subsequent "second-look" surgery or at autopsy.

DISCUSSION

This follow-up report represents a prospective clinical pilot study. During the course of our investigation, we observed microscopically that a glioblastoma could be destroyed completely by irradiation under hyperbaric oxygen, probably at dosages higher than 4,000 rads in $3^{1}/_{3}$ weeks in the TIW schedule (or 1,650 rets) under hyperbaric oxygenation. The autopsy material of 1 such patient, who died 7 months after hyperbaric oxygen therapy, showed a complete disappearance of tumor cells in the irradiated postoperative tumor field. However, the radionecrosis of the adjacent normal brain tissue was extensive.

We have also observed histologically proved recurrent tumor nodules in the previously irradiated field in a few patients who had clinical recurrence of glioblastoma and who subsequently underwent a second-look operation.

Glioblastoma is one of the most malignant and rapidly fatal tumors. A 5-year survivor of glioblastoma is an exception rather than the rule. Perhaps an incorrect initial diagnosis was made in those 5-year survivors, either because of a mis-sampling and misinterpretation of a lower grade astrocytoma (e.g., grade II as grade III) or because of a slow-growing variant of the malignant tumor.

For assessment of the response of glioblastoma to a new treatment technique, it has been our policy to start with a dosage system that ranged from a lower dose upward, instead of from the higher range downward, so that any untoward normal tissue damage can be detected early and the technique modified. In the dose system used, we encountered few untoward reactions until a dosage of 4,400 rads in $3^2/_3$ weeks (400 rads TIW) was reached. Subsequently, we changed to a schedule of 6,000 rads in 6 weeks (200 rads/treatment and 5 treatments/week). The initial 3,000 rads are given in air and the subsequent 3,000 rads under hyperbaric oxygenation. The rationale is based on the new radiobiologic evidence that partial reoxygenation of hypoxic tumor cells indeed takes place soon after conventional daily irradiation. It is conceivable that only the most resistant residual hypoxic tumor cells in the core of the tumor would require hyperbaric oxygen for their ultimate elimination in the last part of the course of radiotherapy. Atkins et al. (*11*) noted that the huge size of the dose per fraction in the one- or two-fraction treatment schedule accounts for dramatic radiation injury of the normal tissues. The injury is more pronounced in the hyperbaric oxygen than in the control group. Whereas the size of dose per fraction is reduced to 333 rads or less, the number of fractions is increased to 12 or 15, and the total dose stays within the isoeffect dose range of 1,600–1,800 rets, the brain tissue injury by irradiation was greatly reduced and acceptable, either treated under hyperbaric oxygen or control conditions (Chang CH, Herbert C Jr, Mastri A: Manuscript in preparation).

Because the state of the art for direct measurement of brain tissue oxygen tension is still unsatisfactory, we did not subject our patients with glioblastoma to this technique. However, the cerebral vasoconstriction upon administration of hyperbaric oxygen may not pose a serious problem for delivering molecular oxygen to the tumor cell, as shown by the experiment of Ledingham et al. (*9*); the value of cerebral blood flow is not reduced while pure oxygen is being inhaled at 3 atm. Hyperbaric oxygen may induce hypocapnia, which in turn may divert and facilitate cerebral blood flow from constrictive cerebral vessels to the dilated tumor vessels (*10*).

Of those who survived beyond 30 months, 1 died of cystic tumor recurrence, and the tumor, histologically, was a grade IV astrocytoma, with coagulation necrosis at some areas. Another died of a recurrent large cystic tumor with microscopic evidence of tumor transformation from a grade III to grade IV astrocytoma. The 2 patients who survived beyond 45 months were still alive in February 1976; one suffered from a grade III and the other from a grade IV astrocytoma; both were treated under hyperbaric oxygen with daily fractionation schemes and had laboratory evidence (radionuclide brain and computerized tomographic scans) of a small residual mass or necrosis but were neurologically and functionally intact with few symptoms.

The survival rate for the oxygen group as compared with that of the controls is not statistically significant. However, the marked difference in the median survival time between those 18 patients who were irradiated with the conventional daily fractionation scheme under hyperbaric oxygen and 15 controls was sufficiently encouraging to warrant further controlled clinical trial.

If a radiation adjuvant is to have an important effect on radiotherapy results, it must demonstrate significant improvement when compared with the best results achieved with current available techniques. With our pilot study on varying dose fractionation from unconventional 3 fractions/week to conventional daily treatments of 6,000 rads in 6 weeks, we found that hyperbaric oxygen may be an effective adjuvant to clinical radiotherapy for glioblastoma with the conventional daily fractionation scheme. This pilot clinical study paved the way for further controlled clinical trial of oxygen and oxygen-mimicking drugs, including the electron-affinic compounds (*14*), which may differentially sensitize the hypoxic tumor cells.

ADDENDUM

At the time this galley proof was read, the 2 patients in the oxygen group were still alive and ambulatory beyond 5 years, and latest CT scans showed no evidence of tumor recurrence.

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Current Status of Intrathecal Chemotherapy for Human Meningeal Neoplasms[1,2]

W. Archie Bleyer[3]

ABSTRACT—Progress in clinical intrathecal chemotherapy has been slow, and a number of new problems have been encountered. The most significant recent developments include the: 1) success of adjunctive intrathecal chemotherapy in the prevention of meningeal leukemia; 2) recognition and characterization of a necrotizing leukoencephalopathic reaction associated with intrathecal methotrexate therapy; 3) demonstration of the efficacy of intraventricular chemotherapy via an Ommaya reservoir; 4) delineation of the clinical pharmacology of intralumbar and intraventricular methotrexate; and 5) identification of an urgent need for new drugs and techniques for intrathecal administration.—Natl Cancer Inst Monogr 46: 171–178, 1977.

During the past decade, meningeal neoplasms have become a common concern of neurosurgeons, neurologists, radiation therapists and medical oncologists, largely because the number of patients with central nervous system (CNS) metastases from systemic cancer has increased. One explanation for this increase is that the risk of CNS infiltration in the patient with systemic cancer appears to be directly proportional to the duration of survival. As survival has increased, so has the incidence of CNS metastases. Successes in the treatment of systemic cancer have thus created an even greater need for effective therapies of CNS neoplasia.

This paper summarizes the major developments in intrathecal chemotherapy since the reviews on this topic by Bering et al. (*1*), Shapiro and Ausman (*2*), and Broder and Rall (*3*). The rationale, limitations, and problems of human intrathecal chemotherapy will be discussed, and some of the recent advances and new approaches to these problems will be reviewed. Because methotrexate (MTX) is used for the vast majority of intrathecal drug administrations in man, intrathecal MTX therapy will be emphasized.

RATIONALE

The administration of drugs directly into the cerebrospinal fluid (CSF) is an old concept based on the rationale of rapidly bypassing the blood–brain barrier and achieving a high concentration of an active agent where it is most needed. Even without the blood–brain barrier phenomenon, the intrathecal route may provide a high concentration within the CNS with less risk of toxicity than the intravascular route. This is true for drugs that are rapidly detoxified after they enter the blood stream and also for those avidly bound to the plasma proteins. Because the initial volume of distribution after intrathecal injection is much smaller than the corresponding volume after systemic administration (e.g., 140 ml for CSF vs. 3,500 ml for plasma), the intrathecal route offers a greater potential for high concentrations of the drug in the CNS. Also, the half-disappearance time of a drug is significantly greater in the CSF than in the systemic circulation [e.g., the initial half-life for MTX is 4.5 hr in the CSF (*4*) and 45 min in the plasma (*5*)]. Even intracarotid injection could not achieve the concentrations reached with the intrathecal route, partly because the circulation time from artery to vein is less than 10 seconds, whereas the CSF circulation time may be as long as 4 or 5 hours.

After a drug is injected into the CSF, its distribution within and removal from the CNS is affected by a number of processes: 1) bulk flow of entrained drug via the normal pathways of CSF flow, 2) diffusion into and through the extracellular fluid of brain parenchyma and spinal cord, 3) back diffusion from the extracellular fluid of brain parenchyma into the CSF and removal from the CNS by the normal pathways of CSF absorption and by diffusion from the extracellular fluid into the capillaries of the brain and spinal cord. Other processes may include: 4) diffusion into the arachnoid and dural membranes and removal via the circulating blood of the meninges; 5) absorption from ventricular fluid by an active transport mechanism located in

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Supported in part by Public Health Service grant CA-16525 from the National Cancer Institute, Bethesda, Md. 20014.

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the choroid plexus, and 6) uptake by certain cells in the brain and spinal cord. To achieve a high concentration at the site of the tumor, little loss of drug must occur as it moves along the CSF pathways to the site overlying the tumor and then diffuses into the CNS parenchyma. Thus the ideal agent is one that is cleared slowly from the CSF, diffuses rapidly through the extracellular space, and undergoes little, if any, uptake by the glial cells, neurons, and capillaries within the CNS (*6*). The drug must also be tumoricidal against the neoplasm, and it should have no significant neurotoxic side effects.

LIMITATIONS AND TOXICITIES

One of the more urgent problems of current intrathecal chemotherapy is that MTX and cytosine arabinoside (ara-C) are the only antineoplastic agents that are available commercially for intrathecal therapy in man. Other agents have been tested but these have proved to be too neurotoxic (e.g., 5-fluorouracil, radioactive gold, adriamycin) or no more effective than iv administration (e.g., asparaginase).

Another concern is whether intrathecally administered drugs can reach brain tumors located several millimeters or centimeters from the CSF–brain interface. The consensus has been that the effectiveness of intrathecal chemotherapy will be limited to tumors located adjacent to or contiguous with the CSF pathways, i.e., leptomeningeal tumors, ependymomas, or to tumors that spread or metastasize via the CSF, e.g., medulloblastoma. Recent studies performed in the rhesus monkey by Blasberg et al. (*7*) tend to support this conclusion. After a 1-hour ventriculocisternal perfusion, these investigators measured the drug concentration in brain tissue as a function of the distance from the ependymal surface. The distance into the brain at which the drug concentration was 1% of the CSF concentration was 3.2 mm for MTX, 1.8 mm for ara-C, 1.5 mm for triethylenephosphoramide (thio-TEPA), and 1.4 mm for 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU).

Another problem is that the two most commonly used intrathecal drugs, MTX and ara-C, are phase-specific, cycle-dependent cancer chemotherapeutic agents. Their principal mechanism of cytotoxicity is inhibition of DNA synthesis. As a result, these drugs are most effective against rapidly proliferating tumors, in which many of the cells are progressing through the phase of DNA synthesis (S-phase). The cytotoxic effect of such drugs would be compromised by most primary brain tumors, in which few of the tumor cells are in S-phase at any one time (*8*). Also, the cytokinetics of a neoplasm metastatic to the CNS may differ significantly from the kinetics of the tumor at its primary site. For example, there is some evidence that leukemia cells may proliferate more slowly in the CSF than in the blood or bone marrow. In a study of 3 patients with stable meningeal leukemia, Kuo et al. (*9*) observed that only 21–55% of the malignant cells in the CSF progressed through the cell cycle over a 10-day interval. They also observed that less than 0.1% of the cells were in S-phase at any one time and that about 1% of the cells were in mitosis. If confirmed in other patients, these characteristics will have major implications for the duration and kinds of chemotherapeutic agents to be used.

Some of the variability in therapeutic and toxic effects can be explained by the site of injection which, for most drug administrations in man, is the lumbar puncture. First, as many as 11–24% of the drug administrations may be injected inadvertently into the epidural or subdural space rather than into the subarachnoid space (*10, 11*). Second, the lumbar puncture may create a leak in the meninges and thereby alter the flow, pressure, and volume of the CSF, as well as result in lumbar puncture syndrome. Third, the drug must ascend the spinal subarachnoid space against the briskly descending bulk flow of CSF, as recently demonstrated by Di Chiro et al. (*12*). Absorption of the drug along the spinal pathway may diminish significantly the amount of drug reaching the cisterns at the base of the brain. Finally, to enter the cerebral ventricles, the drug must overcome the nearly unidirectional flow of CSF in the opposite direction through the aqueduct of Sylvius and ventricular outlet foramina. Shapiro et al. (*13*) and Bleyer et al. (*14*) demonstrated recently that this may be a significant problem with intralumbar MTX therapy. These investigators measured the concentrations of MTX in ventricular CSF after injection of the drug into the lumbar subarachnoid space and found that not only were the concentration–time profiles widely variable, but the peak concentrations were generally low relative to the drug levels needed for a significant antitumor effect.

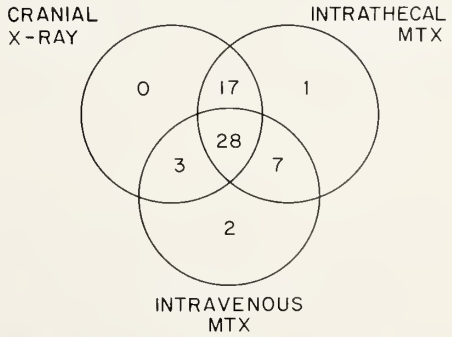
Also, the tumor may interfere directly or indirectly with these processes and alter the distribution and concentration–time profile of the drug within the CNS. For example, CSF flow may be altered by obstructions in the subarachnoid space or ventricular pathways by tumor or adhesions. The presence of leukemia cells in the CSF has also been associated with significant elevations of MTX concentration in the CSF (*15*). A high protein content in the CSF may increase protein-binding of the drug and diminish the amount of free drug available for transport into the tumor cells.

The toxicities of intrathecal chemotherapy also have been a major problem. Toxicity to systemic tissues depends on the type of drug and the dosage administered. In general, the systemic toxicity of intrathecal ara-C is minimal, probably because the drug is metabolized as rapidly or almost as rapidly as it leaves the CNS. However, with intrathecal MTX, systemic toxicity is potentially greater than with the same dose given systemically. Jacobs and his associates (*16*) have shown that MTX concentration in plasma remains above toxic levels for significantly longer intervals after intrathecal injection than after iv or oral administration of the antifolate. This finding was attributed to a reservoir effect of the CNS, with a slow release of the drug into the systemic circulation and a more sustained plasma level at toxic concentrations. Although systemic toxicity of intrathecal MTX can be prevented effectively with systemic therapy with citrovorum factor, recent evidence shows that citrovorum factor can augment the levels of reduced folates in the CNS and thereby possibly neutralize the effectiveness of MTX in the CNS (*17*).

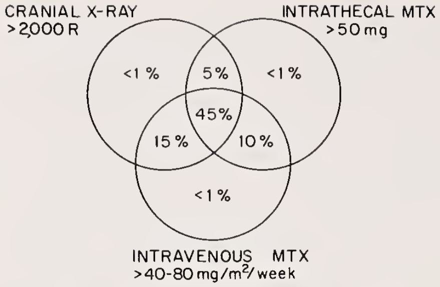
The most distressing complication of intrathecal MTX therapy has been its neurotoxicity, which develops in three clinical patterns: acute, subacute, and delayed (or chronic). The most common form of toxicity is chemical arachnoiditis, an acute syndrome that begins within hours after injection and subsides within 1–5 days. The signs and symptoms are those of meningeal irritation and increased intracranial pressure: headache, backache, vomiting, fever, and meningismus. The CSF reveals a granulocytic pleocytosis and the findings can mimic bacterial meningitis (*18*). The severity of this reaction appears to be related to the dose of MTX administered, to the peak MTX concentration in the CSF, and to alterations in the osmolality and pH of the injected solution (*15*, *19*). Intrathecal ara-C has also been associated with acute arachnoiditis (*20*), although it occurs less commonly than with MTX.

The subacute form of intrathecal MTX neurotoxicity occurs a few weeks after therapy is initiated. It is characterized primarily by motor dysfunction of the brain or spinal cord. The most frequent symptoms are paraparesis, cerebellar dysfunction, and cranial nerve palsies. Paraplegia, quadriplegia, seizures, and obtundation also have been reported (*21*). This pattern occurs most frequently in patients receiving 2 or more injections per week and is apparently related to a continuously elevated concentration of MTX in the CSF (*15*).

The delayed form of neurotoxicity, necrotizing encephalopathy, occurs months to years after the onset of intrathecal MTX therapy and only recently has been recognized as a distinct syndrome related to MTX therapy (*22–27*). The clinical pattern is one of progressive neurologic deterioration beginning insidiously and evolving into severe dementia, dysarthria, dysphagia, ataxia, spasticity, seizures, and coma. Thus far, descriptions of 58 cases, consistent with the syndrome, can be found in the literature (*21–34*), and in nearly every instance of the 48 autopsied, the lesions were identical: diffuse multifocal coagulative necrosis, demyelination, and axonal swelling of white matter. In every instance, the patients had been treated previously with more than 2,000 rads of irradiation to the cranial vault, more than 50 mg MTX/m^2 intrathecally, and/or frequent intensive systemic MTX therapy. Thus three factors (cranial irradiation, intrathecal MTX, and systemic MTX) have been implicated in the pathogenesis of this toxicity. When analyzed by distribution according to therapy (text-fig. 1), it becomes clear that most of the reported cases have occurred in patients administered two or three of these therapeutic modalities. The actual risk is difficult to ascertain, but an approximation of the frequency of leukoencephalopathy can be made from the published series (text-fig. 2) For example, crippling encephalopathy was observed in 9 of 20 patients treated by Aur et al. (*28*) with all three forms of therapy. Meadows and Evans (*30*) observed severe encephalopathic reactions in 4 of 23 long-term survivors treated with systemic MTX. Smith (*29*) examined the brains of 10



TEXT-FIGURE 1.—Distribution according to therapy of the reported cases of leukoencephalopathy.



TEXT-FIGURE 2.—Risk of leukoencephalopathy according to therapy. The figures on risk, which were estimated by the author from a review of the literature, should be interpreted as rough approximations.

patients administered intrathecal MTX with or without irradiation and found white matter necrosis in 3, all of whom apparently died as a result of this complication. Postmortem evidence of leukoencephalopathy was noted by Bresnan et al. (27) in 6 of 9 patients with brain tumors treated with intraventricular MTX and cranial irradiation.

Apparently, the risk for leukoencephalopathy depends not only on the combination and permutation of the therapeutic modalities, but also on the amount of therapy given. For example, Price and Jamieson (*23*) found a direct correlation between the incidence of leukoencephalopathy and the cumulative dose of MTX. Rubinstein et al. (*24*) reported a 47% incidence of leukoencephalopathy among patients treated with a higher dose of cranial irradiation (>3,500 rads) and a larger total dose of intrathecal MTX (>150 mg). Their study also suggested that other cytotoxic drugs may contribute to the risk because their patients also were treated with intrathecal ara-C and intrathecal hydrocortisone. The large number of young children among the reported cases suggests that age may also be a determinant and that the maturing brain of a young child may be more vulnerable to this toxicity. The underlying tumor does not seem to be a primary determinant, for leukoencephalopathy has been observed with various neoplasms of the CNS and, disturbingly, 14 of the reported cases received "prophylactic" CNS therapy.

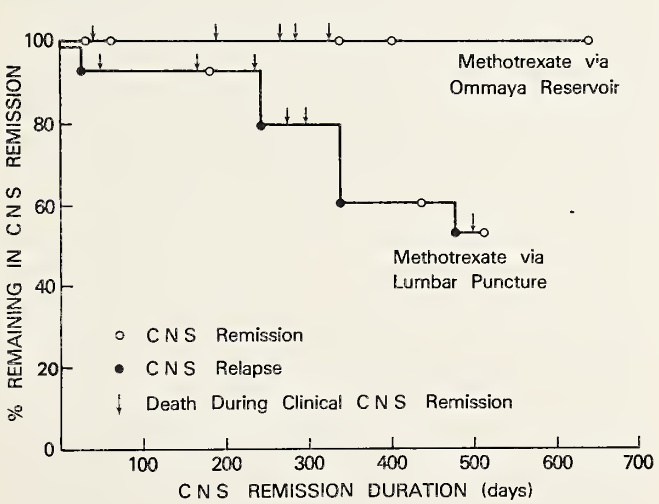
RECENT ADVANCES AND NEW APPROACHES

The problems of intrathecal chemotherapy are being solved slowly. The indications and timing of intrathecal chemotherapy have been clarified recently by pathologic studies on the growth and spread of tumors within the human CNS. Price and Jamieson (*23*) have shown that early meningeal leukemia is limited to the arachnoid membrane. As the disease progresses, the tumor cells obliterate the CSF pathways, penetrate the pia-glial membrane, and eventually invade the deeper layers of the brain parenchyma. Shapiro and his co-workers (*35*) noted a similar pattern in meningeal carcinomatosis. Also, certain primary brain tumors, e.g., medulloblastoma and ependymoblastoma, have metastasized via the CSF. These findings indicate that intrathecal chemotherapy will be more effective in some tumors than in others, and that it will be most effective if administered early in the course of the patient's disease. Ideally, it should be given "prophylactically" or adjunctively before the disease becomes clinically apparent.

Another improvement is the concept of continuing therapy for a proscribed number of courses after an apparently complete clinical remission has been achieved. This concept of "consolidation therapy" is based on the probability that a large number of tumor cells remain in the CNS at this time despite a negative CSF cytology. In meningeal leukemia, Selawry and Odom (*36*) have estimated that at least 10^5 tumors remain in the CSF at the time the chamber cell count and differential white count return to normal. They reasoned that a minimum of five additional injections of intrathecal chemotherapy would be necessary to eradicate the tumor cell population. The best evidence in support of this hypothesis comes from the study of Sullivan et al. (*37*) on the treatment of overt meningeal leukemia. These investigators randomized patients between a "no-therapy" maintenance regimen and one in which intrathecal MTX was continued at 6- to 8-week intervals. The results were striking. The median duration of CNS remission was approximately 12 months in the continuation therapy group and about 3 months in the unmaintained group, all of whom eventually relapsed, whereas 10 of 19 patients on the continuation therapy regimen had no recurrence and 4 have been in long-term CNS remission. Another reason continuation therapy may be effective is that it may prevent reseeding of the CNS by tumor cells in the blood.

The most significant clinical advance in intrathecal chemotherapy has been the use of an Ommaya reservoir to administer chemotherapy intraventricularly. A number of reasons indicate why intraventricular administration should be better than intralumbar injection: The chemotherapist can be certain that he has injected the drug into the CSF; the distribution of the drug in the CSF will be maximized since the cerebral ventricles are the site of CSF production, and entrained drug will be carried by bulk flow throughout the CSF compartments (*7*). Also, the reservoir chamber permits more frequent injections with less patient discomfort than spinal injections via lumbar puncture.

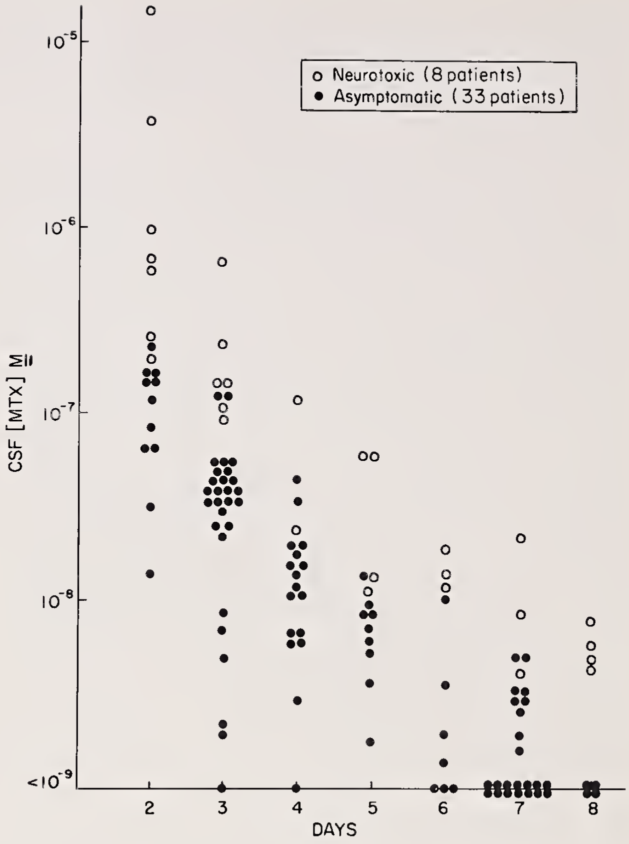
That intraventricular injection significantly improves intrathecal chemotherapy of meningeal neoplasms has now been shown by Shapiro et al. (*35*) at the Memorial Sloan-Kettering Cancer Center. Between 1970 and 1974, they implanted Ommaya reservoirs in 67 patients with active meningeal neoplasms. Thirty patients had solid tumors (15 breast cancer, 7 lung cancer, 5 melanoma, and 3 other tumors), 7 had lymphoma, 22 had meningeal leukemia, and 8 had leukolymphoma. MTX (6.25 mg/m^2) was injected into the reservoirs at various intervals but usually twice weekly initially, then less frequently. Overall, 79% of the 62 adequately treated patients improved. Nine children with meningeal leukemia were treated first by lumbar puncture and later by intraventricular injection. In these 9 patients, 34 episodes of meningeal leukemia occurred in 142 patient-months during lumbar treatment and 7 episodes (2 of which were equivocal) in 112 patient-months during intraventricular treatment. The number of episodes per month in the ventricular treatment period was one-fourth that in the lumbar treatment period ($P < 0.01$). We observed a similar pattern in studies performed at the Pediatric Oncology Branch of the National Cancer Institute (NCI). During the last 4 years, two treatment programs for overt leukemia of the CNS were conducted in which intrathecal MTX was the only CNS therapy: protocols NCI 725.1 (1972–73) and NCI 736.2 (1974–75). In both studies, 12 mg MTX/m^2 was given weekly for 6 doses, then monthly thereafter. The only difference was the route of administration. MTX was administered by lumbar puncture in the earlier study and intraventricularly in the current one. When each study was analyzed 2 years after initiation (i.e., at the same follow-up time), the group having the Ommaya reservoirs showed a greater therapeutic effect ($P<0.07$) as illustrated in text-figure 3. These results are even more impressive when differences in prognostic factors between the 2 groups are considered. Five of the 10 patients in the Ommaya reservoir protocol had had an episode of overt leukemia of the CNS prior to entering the study as compared with 1 of the 13 patients on the lumbar puncture protocol (*38*).



TEXT-FIGURE 3.—Intraventricular vs. intralumbar MTX for treatment of leukemia of the CNS. Results depicted represent NCI protocols 736.2 (*upper curve*) and 725.1 (*lower curve*).

Although a high incidence of complications related to the insertion and use of the Ommaya reservoir have been reported (*39*), toxicity in the studies described above has been acceptable. In the study by Shapiro et al. (*35*), complications occurred with 20 (18%) of 110 reservoirs. The cannula tip was misplaced in three and became occluded in six. Transient or persistent focal neurologic deficits occurred in 3 patients and 1 patient had a postoperative pulmonary embolism. Infection by culture developed in 7, only 1 of whom had clinical meningitis; in 4, the device was saved by the administration of antibiotics. In our study (*38*), 2 of 23 patients had clinically significant complications related to the reservoir. One patient had a subdural hematoma and another developed staphylococcal meningitis, which was treated with intraventricular and iv methicillin. The reservoir was not removed and, 1 year later, continues to function well without recurrence of infection. The low complication rate can be attributed to several factors. Infections have been reduced by strict adherence to sterile technique and by the use of a small-gauge, scalp-vein ("butterfly") needle for injections of the reservoir. We eliminated neurologic deficits induced by reservoir implantation by inserting the reservoir transfrontally instead of transoccipitally. The cannula tip has not been misplaced since fluoroscopy was added to the implantation procedure (*35*). That neurosurgical expertise is imperative is illustrated by the first patient in our series. The skin incision was located directly over the reservoir chamber rather than off to the side. Skin tension over the bulge of the reservoir chamber forced a sc suture from beneath the incision to erupt through the skin; the incision and reservoir then became infected, and the patient developed staphylococcal meningitis.

A number of new methods reduce the neurotoxicity of intrathecal chemotherapy. Sullivan (*40*) has demonstrated that the simultaneous administration of intrathecal hydrocortisone sodium succinate decreases the severity of the acute toxicity (arachnoiditis). Geiser et al. (*41*) have attenuated the acute toxicity of intralumbar MTX by diluting the antitumor drug in Elliott's B solution before injection. Elliott's B solution is an artificial CSF, and the resulting solution is more physiologic with respect to toxicity and pH (*42*). Another technique is to adjust each patient's dosage relative to the concentration of the drug in his CSF. This modification is predicated on the observation that the acute and subacute neurotoxicities of intrathecal MTX are associated with elevated concentrations of the drug in the CSF (text-fig. 4). During the past 2 years, we have adjusted each patient's intrathecal dosage relative to the MTX concentration in his CSF, increasing the amount in patients with low levels and decreasing it in those with high levels. Since the institution of pharmacologic monitoring and individual dosage modification, we encountered only 3 patients (6%) with neurotoxicity among the 50 treated, compared with 21 (38%) of the 56 patients given intrathecal MTX during the 2 previous years ($P < 0.05$) (*15*). Of the 3 patients with neurotoxicity despite dosage modification, 2 had arachnoiditis with the first dose and 1 developed leukoencephalopathy 3 months after starting therapy (*33*). Use of the MTX concentration in CSF as a therapeutic guide seems to reduce the incidence and severity of the acute and subacute forms of neurotoxicity.



TEXT-FIGURE 4.—MTX concentrations in CSF in 41 patients treated with intralumbar MTX. *Solid dots* represent 33 patients with no evidence of neurotoxicity. *Open circles* represent 8 patients with serious neurotoxicity: 1 with grand mal seizures, 1 with quadriplegia, 1 with cerebellar dysfunction, and 5 with severe arachnoiditis. Antifolate levels in the neurotoxic patients were significantly higher than corresponding values in the asymptomatic patients.

Obviously, new drugs and methods are needed to improve the therapeutic index of intrathecal chemotherapy. An alkylating agent, thio-TEPA, which has entered clinical phase I trials (*43*), is particularly promising because its antitumor effectiveness is not limited to the S-phase of the cell cycle. The intrathecal injection of a combination or sequence of drugs is another promising approach. Combining ara-C with MTX in the correct sequence and interval may increase the therapeutic effect (*44*). Another possibility is to combine a phase-specific agent (MTX or ara-C) with a cycle-nonspecific drug (thio-TEPA). Administration of chemotherapy is a heavier-than-water solution, e.g., 10% dextrose, and placement of the patient in the Trendelenburg position may promote better distribution of the drug from the lumbar space to the cerebral subarachnoid space and thereby also may decrease spinal arachnoiditis and spinal cord dysfunction (*45*). New dosage schedules also are being evaluated; e.g., a "concentration × time" MTX schedule has been devised to maintain a relatively constant cytocidal concentration for 72 hours (*38*). This regimen delivers one-third to one-half the total drug administered on a conventional schedule. Another approach that appears promising is ventriculolumbar perfusion, in which an MTX solution is infused over a 4- to 6-hour period via an Ommaya reservoir, and CSF is drained simultaneously from a lumbar cannula (*46*). This technique improves drug distribution within the CNS, essentially replacing the entire CSF with the MTX-containing solution.

One problem that may soon be solved is whether intrathecal chemotherapy can ever be used effectively against brain tumors located several millimeters or centimeters from the CSF–brain interface. Here the major issue is whether tumoricidal drug concentrations can be achieved at the site of a tumor. Recently, Blasberg and his associates (*47*) have applied their observations in nonhuman primates to intrathecal MTX therapy in man. Using known concentrations of MTX in human CSF after intraventricular injection of 6.25 mg/m^2, they calculated that MTX would reach a brain concentration of 10^{-6} M 7–20 mm from the ependymal and pia-glial surfaces. Since most of the human brain is within 10 mm of CSF, these predictions provide a rationale for the use of intrathecal chemotherapy in brain tumors other than meningeal neoplasms. Another technique that might enhance penetration of the drug into the tumor is to simultaneously administer the drug intrathecally and in high doses iv. This approach recently has been applied successfully in the prevention of meningeal leukemia (*48*).

Eventually, intrathecal chemotherapy may be replaced by systemic administration of antitumor drugs with optimal physiochemical characteristics for passage into the CNS. Meanwhile, intrathecal chemotherapy will be important in the treatment of CNS neoplasms and, it is hoped, some of the new approaches will improve substantially this therapeutic modality.

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Treatment of an Intracerebral Rat Brain Tumor With *Corynebacterium parvum* and Radiation[1,2]

Evan B. Douple[3,4]

ABSTRACT—Rats bearing intracranial brain tumors were treated with single ip injections of the killed *Corynebacterium parvum*, a single dose of X-rays, or a combination of both treatments. Animals given injections of 2.6 mg *C. parvum* 12 days after implantation of the tumor had a median survival time (MST) of 50 days compared with an MST of 44 days for an untreated group. These animals given *C. parvum* had a 33% increased life-span (ILS) that was significant at the P=0.05 level. Although the MST of animals that received *C. parvum* 2 days before a tumor X-ray dose of 3,015 rads on day 14 was greater than that of rats given irradiation alone, the ILS of the group administered both treatments was not significant relative to the latter group. The MST of animals that received *C. parvum* 7 days before irradiation was not different from that of animals given injections of *C. parvum* 1 day postirradiation.—Natl Cancer Inst Monogr 46: 179–184, 1977

Human glial tumors are generally radioresistant, and the efficacy of radiation therapy is limited by the damaging consequences of radiation to the normal brain. In view of a general attitude of discouragement with respect to the control and treatment of brain tumors, recent immunotherapeutic advances deserve experimental study with brain tumor models, particularly in combination with radiation therapy.

The use of nonspecific immunotherapy is based on observations that the injection of some bacterial extracts has the ability to stimulate nonspecifically host resistance to tumors. Nonspecific adjuvant therapy may enhance preexisting immunity, stimulate cell-mediated immunity, and stimulate suppressed immunity when used secondary to radiation therapy. Halpern et al. (*1*) demonstrated that the injection of killed *Corynebacterium parvum* results in potent reticuloendothelial stimulation, and several investigators have elucidated the influence of *C. parvum* on both cellular and humoral-mediated immunity. Evidence shows that tumor immunologic processes are not inhibited by the blood–brain barrier (*2–5*). Mahaley (*6*) observed the accumulation of lymphocytes among malignant brain tumor cells.

Since immunopotentiators are presumed to be most effective in hosts harboring a minimal tumor burden, a rationale exists for their use in advanced tumors if combined with cytotoxic agents. However, few studies have yielded information concerning the effect of combined cytostatic agents, such as radiation, with immunotherapy. The present experiments investigate the effects of single injections of *C. parvum* alone or in combination with radiation therapy on the survival time of rats bearing intracerebral brain tumors.

MATERIALS AND METHODS

The rat brain tumor cells were provided by Drs. Paul T. Kornblith and William H. Sweet and their co-workers of the Massachusetts General Hospital. The original tumor, which was induced in a CD®F rat by weekly iv injections of *N*-nitrosomethylurea, was classified as a grade III astrocytoma. The tumors are maintained in our laboratory by serial passage in rats or in culture. The cells were grown in complete tissue culture media in 75-cm^2 plastic tissue culture flasks. The media included basal medium Eagle (Hanks' salts) supplemented with 10% heat-inactivated fetal bovine serum, essential amino acids, vitamins, L-glutamine, and antibiotic-antimycotic. Two days before each experiment, flasks were seeded with cells and, on the day of the experiment, confluent, monolayer cultures were trypsinized and a single cell suspension was prepared in ice-cold Hanks' balanced salt solution.

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[2] Supported by Public Health Service contract N01 CB12087 from the Division of Cancer Biology and Diagnosis, National Cancer Institute.

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Transplantation of the cells was performed according to methods used in the Howard C. Naffziger Laboratories for Neurosurgical Research at the University of California's Brain Tumor Research Center in San Francisco, as described by Barker et al. (7). Adult, male CD®F rats (Charles River), weighing 150–200 g, were anesthetized with sodium pentobarbital (50 mg/kg body weight) and placed in an animal stereotaxic head holder. The scalp was opened surgically, and a hole was drilled at a point to the left of the midline and anterior to the zeroplane. We threaded a plastic screw with a center-drilled opening into the hole in the skull and injected a 0.01-ml suspension containing 4×10^4 cells 3 mm into the central gray matter with a blunt 22-gauge needle attached to a Hamilton syringe. The central hole in the screw was sealed with bone wax to prevent reflux, and the scalp incision was closed with skin clips.

Three experiments were designed to investigate the effects of the administration of *C. parvum* on the life-span of animals bearing brain tumors. In experiment A, 13 animals received a single ip injection of *C. parvum* (2.6 mg in 0.37 ml) on day 12 posttransplantation. The *C. parvum*, obtained from Dr. John Whisnant, consisted of 7 mg dry weight of washed, formalin-killed organisms suspended in 1 ml of physiologic pyrogen-free saline with 0.01% thimerosal as a preservative.

In experiment B, 13 animals received *C. parvum* (2.6 mg in 0.37 ml) on day 12 and a second group of 13 animals did not; both groups received a single tumor dose of 3,015 rads of X-irradiation on day 14. The irradiation was delivered by placing a 1-cm (diameter) cone from a General Electric Maxitron 300 X-ray machine in direct contact with the scalp of the rat at a 21.5-cm focal spot to skin distance (FSD) and with the plastic screw centered in the beam (fig. 1). The perimeter of the cone was modified with lead shielding so that the radiation dose was well defined and limited to a cylinder of 1 cm in diameter. The machine was operated at 140 kilovolt peaks and 20 milliamperes, and the beam included a 3-mm aluminum filter. The effective half-value layer for this beam is 2.7 mm Al. The radiation dose was measured by inserting lithium fluoride (LiF) thermoluminescent dosimeter rods (TLD–100) encapsulated in plastic into rats at

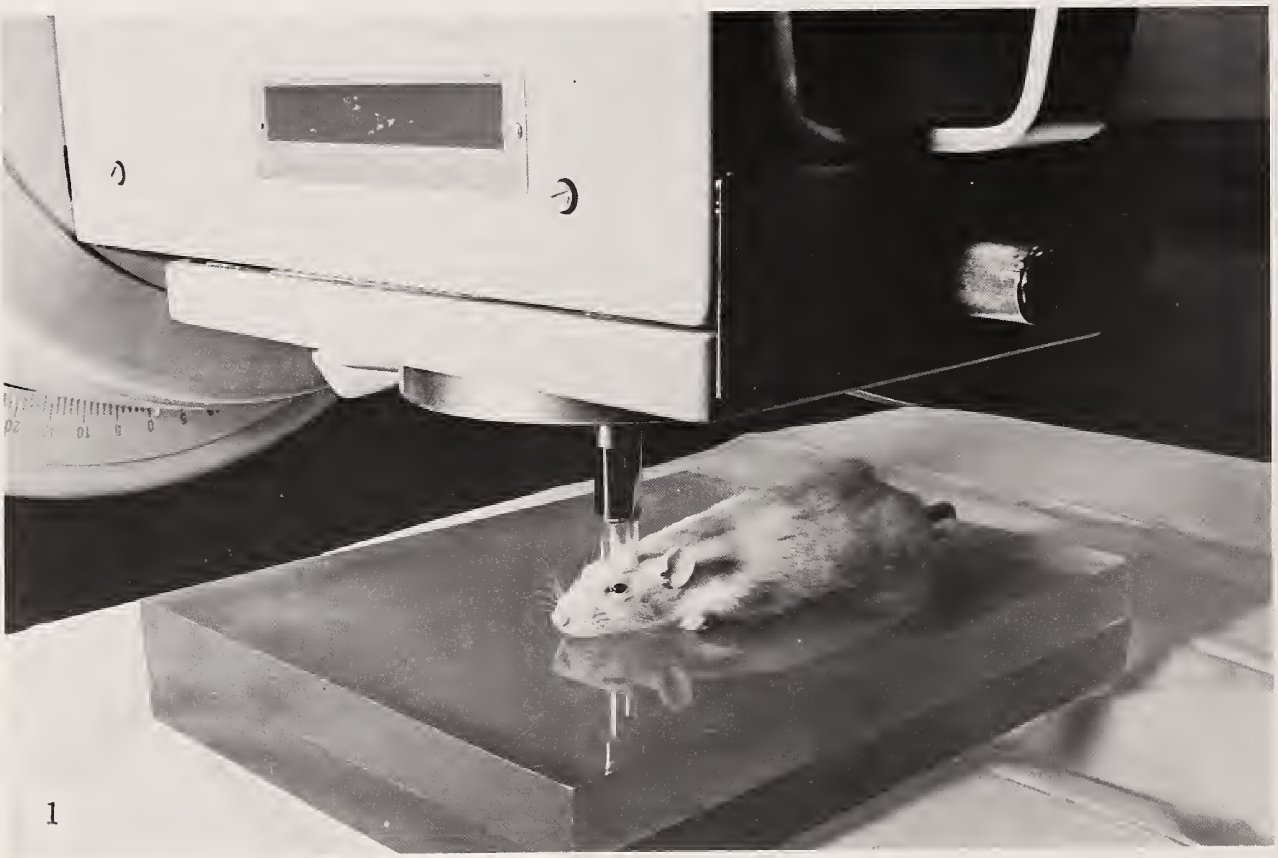

Figure 1.—Irradiation of rat brain with a 21.5-cm FSD and a 1-cm diameter cone.

appropriate monitoring locations (fig. 2). We calibrated the TLD response by using a Lucite phantom and a Baldwin-Farmer ionization chamber. The single 10-minute exposures included a dose rate of 409.8 rads/minute to the rat scalp, 301.5 rads/minute to the tumor, and 172.0 rads/minute to a small section of the mouth and esophagus. The radiation dose of 3,015 rads to the tumor should be sufficient to reduce tumor cell survival levels by a factor of approximately 10^{-4}, based on a recent study in which a similar tumor system was used in conjunction with an in vitro assay as an estimate of in vivo tumor cell survival (*8*).

In experiment C, 26 rats were irradiated with a single tumor dose of 3,015 rads on day 14 postimplantation. Half these animals received a single dose of *C. parvum* (2.6 mg in 0.37 ml) on day 7 and the other half received the bacterium 24 hours after irradiation.

The three experiments included untreated tumor-bearing animals as controls. The animal weights were recorded at various times, and the appearance of the first neurologic symptoms and the days of deaths were recorded. The weights of several internal organs were recorded at autopsy.

RESULTS

Tumor cell growth was observed microscopically approximately 8 days following implantation and macroscopically by day 14. The tumors were well demarcated and confined to the left cerebral hemisphere (fig. 3). Histologically, the tumor was dense, well vascularized, and composed of regions with sarcomatous characteristics. Staining with phosphotungstic acid–hematoxylin did not identify conclusively a significant glial component in the tumor, although some fibers did stain positively. Some evidence of perivascular aggregation of tumor cells outside the primary tumor region was observed. Using a similar brain tumor model, other authors have suggested that similar tumors have become a mixed type, possibly gliosarcomas, after many passages and months of culture in vitro. At the time of the animal's death, the

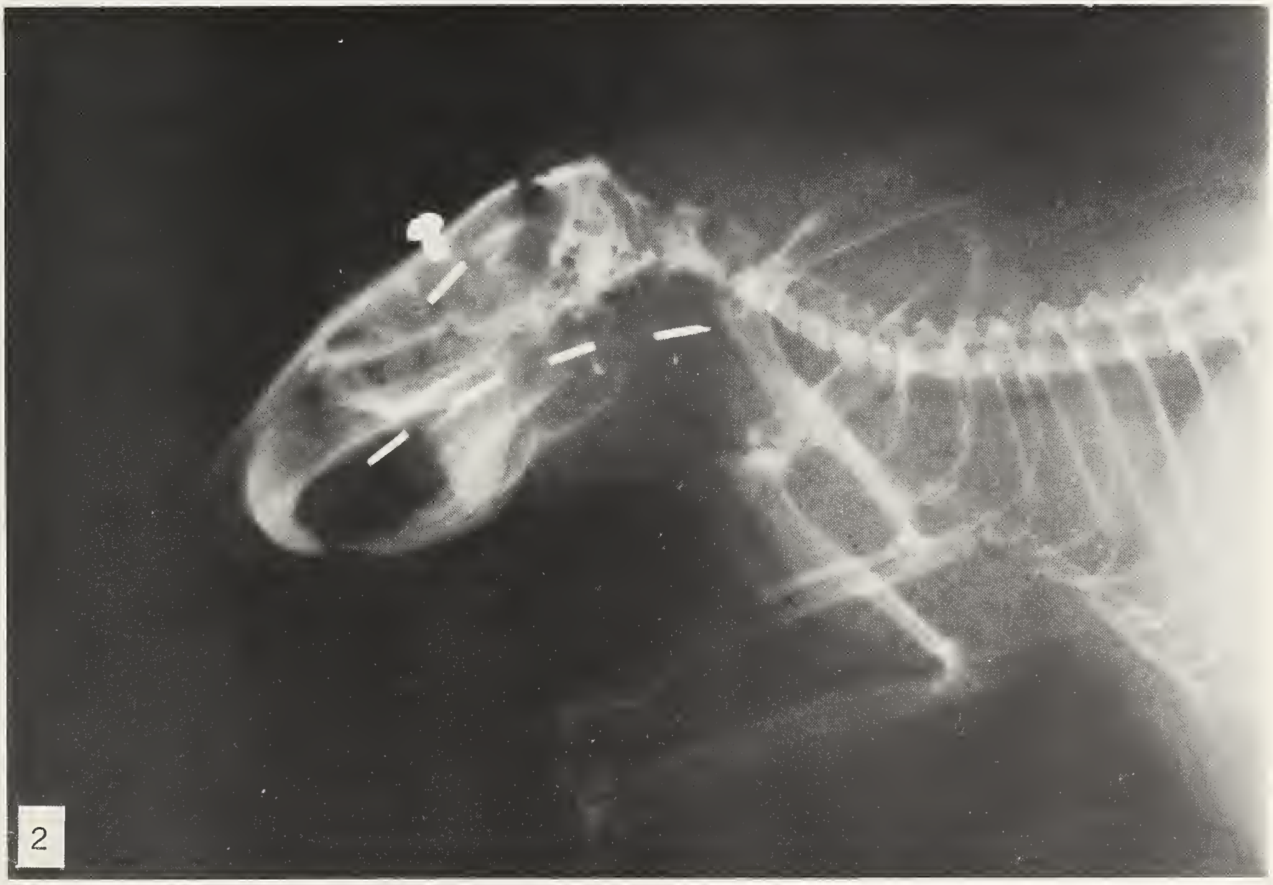

FIGURE 2.—X-Ray of rat showing the location of the brain screw and the TLD-rod monitoring locations at the tumor site and in the mouth. In this X-ray, metal of the appropriate dimensions was substituted for the plastic screw and TLD rods.

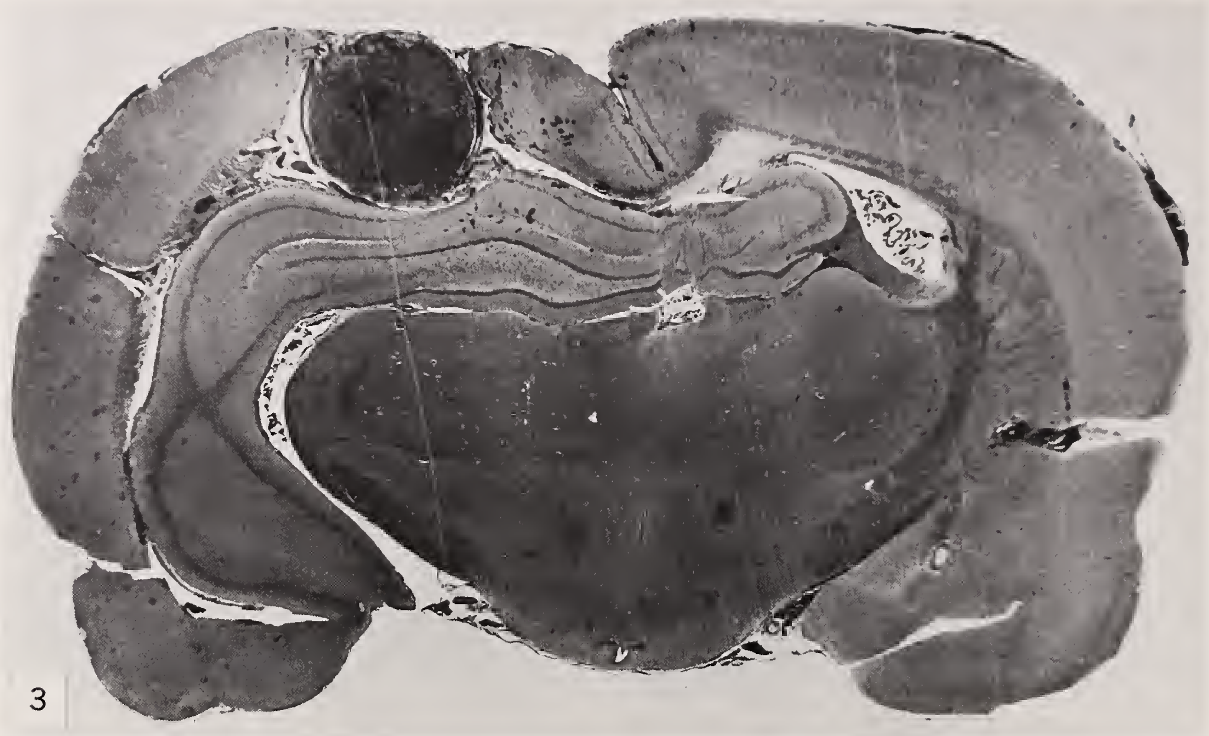

Figure 3.—Cross-section of rat brain 20 days postimplantation containing tumor of approximately 2 mm in diameter. Hematoxylin and eosin.

tumor diameter was approximately 5–7 mm. Death occurs in the rats probably as a result of caudal displacement of the brain stem. In these experiments, the first deaths occurred after 32 days, and the deaths were preceded by a symptomatic period of approximately 6 days, including rapid weight loss, increased tear secretion and red pigmentation around the eyes, and paresis progressing to paralysis. In the 2 days after treatment, the average weights of rats given injections of *C. parvum* decreased by approximately 15 g. The average weights of rats receiving only radiation therapy decreased by approximately 20 g during the 7 days after treatment. Finally, the corresponding decrease in average weights 7 days after the initial treatment was approximately 30 g, when irradiation followed *C. parvum* by 2 days or preceded *C. parvum* by 1 day (text-fig. 1).

The percentage of animals surviving as a function of time postimplantation in the 3 experiments is illustrated in text-fig. 2. Table 1 summarizes the median survival times (MST), the percentage of increased life-spans (ILS), and the ranges of survival times for the 3 experiments. Rats receiving *C. parvum* on day 12 had an MST of 50 days compared with that of 44 days for the untreated group (text-fig. 2A). In this experiment, 2 of the untreated animals were killed 130 days after implantation. Autopsy of the brains of these animals showed no evidence of brain tumors, which suggested that the tumor take in this experiment was not 100%. If these animals are not included in the statistical analysis, the ILS of the animals receiving *C. parvum* is significant ($P = 0.05$) by the Kruskal-Wallis nonparametric test. The survival of 5 animals without neurologic signs in experiment B might not be a result of the therapy, since it is possible these animals did not experience tumor take.

Although the survival curves for the groups of animals receiving the prescribed treatments in experiments B and C appear to be different, the ILS for the groups are not significantly different. It is impossible to compare directly the results of experiments A, B, and C, since each experiment

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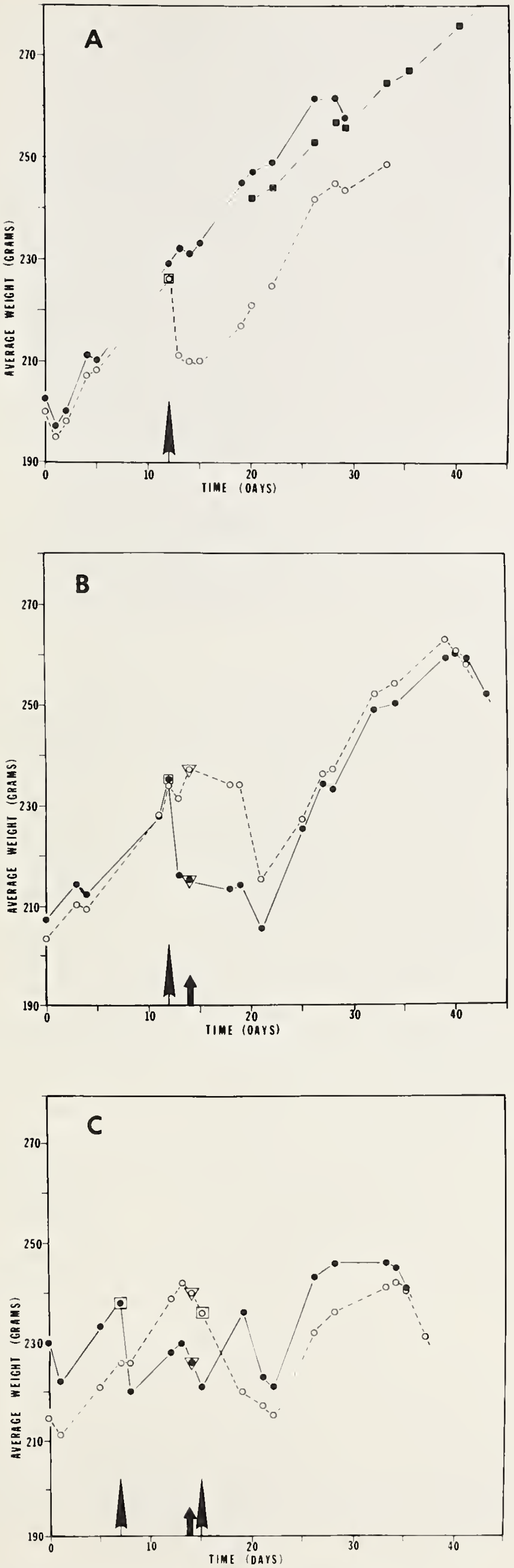
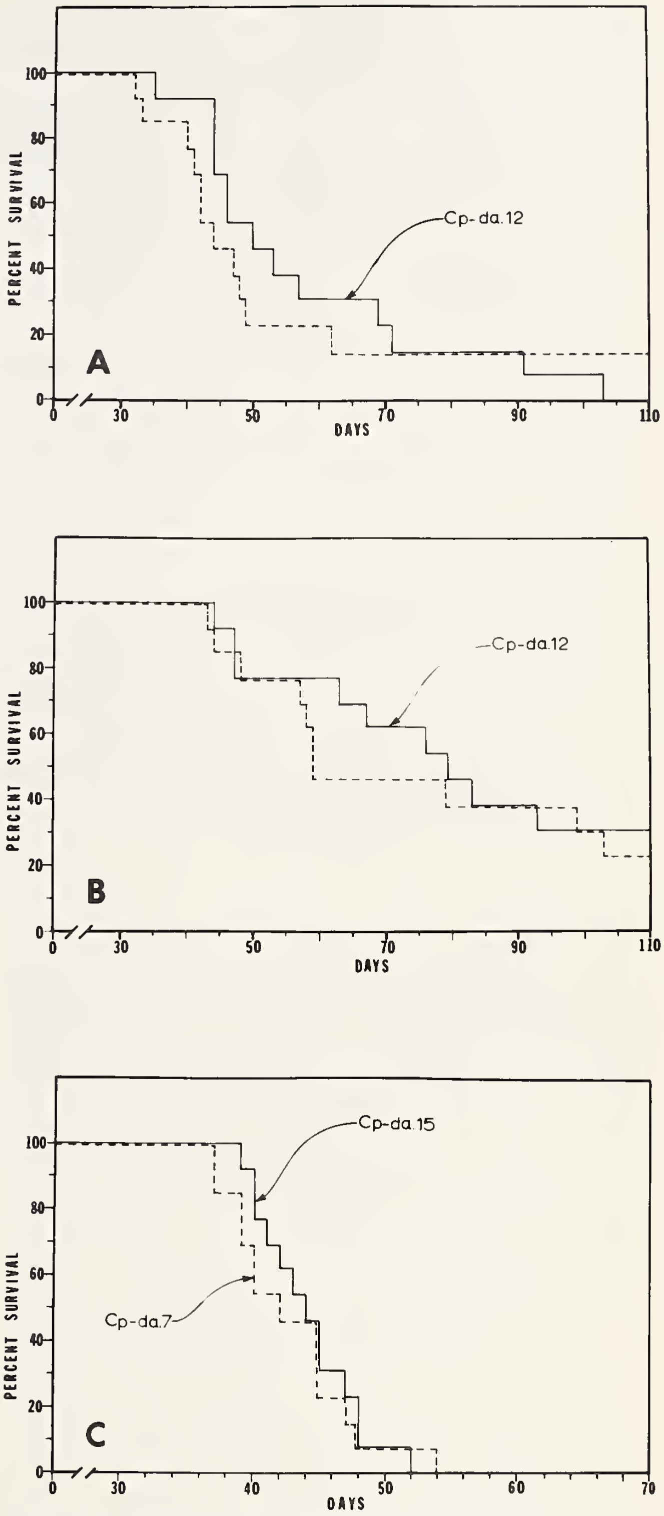
Text-figure 1.—Average weight of animals as a function of time postimplantation. Long *arrows* and *squares* denote days of injection of *C. parvum.* Short *arrows* and *triangles* denote days of irradiation. A) Control animals without tumor (*closed squares*), untreated tumor-bearing animals (*closed circles*). B) Animals receiving *C. parvum* and irradiation (*closed circles*) or irradiation alone (*open circles*). C) Irradiated animals receiving *C. parvum* on either day 7 (*closed circles*) or day 15 (*open circles*).





TEXT-FIGURE 2.—Percentage of animals surviving as a function of time postimplantation. *Dotted line* in A represents the survival of untreated animals. All lines in B and C represent the survivals of irradiated, tumor-bearing rats. *Arrows* denote the curves for animals that received *C. parvum*.

TABLE 1.—*Summary of survival characteristics*

Experiment	Treatment	Day	MST, days	ILS, %	Range, days
A	Untreated		44	—	32—62 [a]
	C. parvum	12	40	33	35—103
B	X-rays	14	59	—	43—130 [b]
	X-rays plus *C. parvum*	14 12	79	8	44—93 [c]
C	X-rays plus *C. parvum*	14 7	42	—	37—54
	X-rays plus *C. parvum*	14 15	44	3	39—52

[a] Two animals were killed at 130 days (without tumor at autopsy).

[b] One animal was killed at 130 days (without tumor at autopsy).

[c] Four animals were killed at 130 days (without tumor at autopsy).

was begun on a different day with a different in vitro culture used for tumor cell implantation. At autopsy, the mean weights of the spleens of animals given *C. parvum* were approximately twice those of the rats that did not receive the killed bacterium. Toxicity studies by other authors have shown that spleen and liver sizes of mice are greatly increased when measured 12 days after a single injection of *C. parvum*.

DISCUSSION

The injection of *C. parvum* at day 12 appears to increase the MST for rats with brain tumors, although the number of long-term survivors did not rise. Using this brain tumor model, we hope additional experiments will determine if larger single doses or multidoses of *C. parvum* will increase this apparent difference in MST. The combination of *C. parvum* with radiation therapy did not significantly alter the survival times obtained in animals receiving radiation alone. However, the sequence for the administration of an immunotherapeutic agent with respect to irradiation and the time intervals between the delivery of the two agents may be critical. Recently, Milas et al. (*9*) and Suit et al. (*10*) have reported greater effectiveness of radiation therapy in the treatment of mouse murine fibrosarcomas if *C. parvum* is administered before irradiation. Currie and Bagshawe (*11*) reported enhanced tumor regression for a solid fibrosarcoma if *C. parvum* is administered 12 days after treatment with cyclophosphamide.

If brain tumor response to radiation therapy can be modified by immunotherapeutic methods, the proper sequence for the application of the therapeutic agents and the timing may be critical and should be investigated. Enhancement of radiation damage is appealing, since radiation therapy is used currently in many brain tumor protocols. Recent improvements in radiologic diagnostic instrumentation may provide precise brain tumor localization. If this improved diagnostic information is combined with computerized treatment planning, it may be possible to deliver higher radiation doses from conventional radiation modalities or charged particle beams to smaller tumor volumes with less risk to adjacent normal tissue. This brain tumor model should provide a system for testing the combination of such potential modifiers of radiation action as immunotherapy, hyperthermia, radiation sensitizers, vitamin A, or anticoagulants with radiation therapy. The results of this pilot study suggest that treatment with the nonspecific immunopotentiator *C. parvum* does not modify significantly the response of an intracerebral rat brain tumor to radiation therapy.

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DISCUSSION IV

J. Ransohoff: Dr. Gutin alluded to the drug interaction, but an interaction also exists between drug and radiation. Many people have asked about the effect of the corticosteroid on radiation therapy. Some have suggested that it may be possible to protect vascular membranes from the steroid. Dr. Chang, in your series, did you have any controls, i.e., those who were not on corticosteroids? Did you compare the steroid dosage in the 2 groups? Dr. Kramer, what are your views on the effect of corticosteroid on radiation therapy?

C. Chang: In our series, all 80 patients received steroid before and after operation without randomization or control. Most patients completed the course of radiation therapy, except a few whose dosage was reduced. I do not know what impact steroids have on radiotherapy. In some cases, we withdrew the steroid early in the course of radiotherapy. Then the patient always complained of tiredness and headaches and sometimes of increased nausea or vomiting. This group of patients had to be put on steroid again, but whether it acts as a general dehydrating agent or has just an anabolic effect, I do not know.

S. Kramer: So far we have no statistical data on the gliomas. After the patient has recovered from a major decompression, I do not maintain him on steroids. We have not seen an antitumor effect in the metastatic tumors. We have seen more rapid subjective improvement in those patients who symptomatically are far advanced, but this improvement is not maintained any longer than in the patients who did not receive steroids. However, the results with steroids have persuaded therapists to give adequate doses from the outset, rather than to start with ineffective doses.

Ransohoff: Is it possible that steroids, by keeping the vascular membranes intact, reduce the long delayed toxic effects illustrated by Dr. Caveness?

Kramer: Yes, that is possible, but we have no solid data.

W. De Wys: In some patients with meningeal disease, especially those with meningeal carcinoma, CSF absorption may be interrupted in a hydrocephalic compartment. Can any advantage be noted, at the time of insertion of the Ommaya reservoir, for simultaneously creating a shunt to the peritoneum or the atrium, so that in the intervals between drug treatment a potential hydrocephalus can be drained?

Dr. Bleyer showed the breakdown of methotrexate CSF levels related to acute toxicity. It seemed to me that the difference is one of initial dose, not one of different kinetics, in the patients with or without toxicity. Could we review the initial dose and set up a new upper limit and, for the most part, avoid toxicity, rather than having to do CSF levels?

A. Bleyer: Ideally, one wants to maintain the drug within the CNS rather than let it pass rapidly into the peritoneal cavity or wherever else the efferent cannula may be put.

De Wys: You can handle that by compressing the outflow.

Bleyer: It can be handled better with an on–off device that permits the therapist to turn off the efferent limb at will. I have tried this approach and think it is likely to work. On the other hand, if one uses both an Ommaya reservoir and one of the ventricular peritoneal shunts separately, i.e., have the Ommaya reservoir on one side and the shunt device on the contralateral ventricle, amazingly high concentrations of methotrexate are retained within the CNS. The only explanation I have is that entry into the contralateral ventricle is not as free as some of the cisternal ventriculograms would suggest and that passage, even when that ventricular peritoneal device is patent, does not shut off the flow in the other ventricle.

Regarding your second point, I agree that in most situations the pattern of higher concentrations is due chiefly to a smaller volume of distribution rather than to a prolonged clearance of the drug from the CNS. The concentrations are higher and the subsequent fall-off is relatively the same; in other words, half-lives are approximately the same, although exceptions do exist. One factor in this difference is shown in the way in which we calculate the dosage for intrathecal chemotherapy. For example, we observe that the older the patient, the higher the concentrations, noticing, by the way, in the old patient that the slope is more shallow and that the half-life is more prolonged. Up to the age of about 40 or 50, the older the patient, the higher the concentrations with approximately the same half-life.

The younger the patient, the lower the concentrations are, perhaps because we calculate the dosage on body surface area. The brain, of course, does not grow with the body surface area, but reaches its adult volume within the first few years of life. The upper dose is 10–12 mg.

G. D'Angio: It has been shown, at least in patients with medulloblastoma, that a shunt placed peripherally increases the metastatic rate outside the CNS. Thus this particular maneuver may not be advisable in patients with established arachnoid disease unless a filter is inserted in the line. Second, in patients with internal hydrocephalus at least, the introduction of methotrexate via the Ommaya device has led to necrosis of the brain. This is, therefore, a potentially hazardous procedure in patients with this complication. Third, we demonstrated by the use of radioactive gold that the flow of that material is helped, if one assumes the appropriate position and uses gravity.

Another mechanism that may lead to difficulty with intrathecal methotrexate, at least when introduced via the lumbar route, is what Rogoff has called the "second sac" syndrome. Repeated lumbar punctures create a second, false subdural sac that can mislead the investigator. He appears to obtain free flows of CSF when he is actually introducing the medication outside the subarachnoid space.

I assume from what Dr. Bleyer said that he uses intrathecal methotrexate only for arachnoidal disease. Does anyone still consider it for solid tumors and, if so, what is the rationale?

Bleyer: It has been recommended that filters be inserted within the efferent cannula in patients with medulloblastoma and other CNS neoplasms that might spread via that route into the peritoneal cavity or wherever the tip is implanted.

Most of the patients who developed paraventricular necrosis had some sort of ventricular outlet obstruction, which suggests that high concentrations were achieved within their ventricles for extended periods of time. We do not know whether intrathecal chemotherapy will be effective for deep-seated brain masses, i.e., tumors some distance from the ependymal or pia-glia membrane. Drs. Blasberg, Fenstermacher, and Potlack studied the concentration of methotrexate at various distances into the ependyma, using the rhesus monkey with a ventriculocisternal effusion, for 1 or 4 hours. They then watched the decay in the stained parenchyma as a function of distance from the ependymal membrane after the infusion is stopped. Some of their data are based on theoretical considerations and some on their experiments in measuring the concentration directly in the brain.

In man, the widest distance between ependyma and pia-glia membrane is 3 cm and that is through the basal ganglia, so it is possible to achieve substantial concentrations of methotrexate deep within the brain. How rapidly is methotrexate taken up by the capillaries that are perfusing the brain? Experimentally and theoretically, it is apparent that methotrexate can enter the parenchyma and move millimeters, perhaps even centimeters, into the brain.

Ransohoff: Dr. Ommaya, do you wish to summarize your 22 years of experience in 3 minutes?

Ommaya: We treated 106 patients with cerebral glioma that we divided into low- and high-grade cases: low grades, 1 and 2; and high grades, 3 and 4. With surgery alone, we had a mean survival of 436 weeks in the low and only 13 weeks in the high grade. All these high-grade cases were large, deep-seated, inoperable thalamic gliomas. Nineteen patients with low-grade gliomas, who were treated with surgery and radiotherapy, had a mean survival of 282 weeks. With radiation therapy alone (and surgery), survival in the high-grade group was equivalent to that of the low-grade group.

Initially, the combined treatment group (surgery, radiotherapy, and chemotherapy) consisted of a few patients who received intrathecal methotrexate by infusion and then perfusion, but most, about 18 patients, received a combination of azaguanine locally, with the use of a tumor-cyst device that maintains a cavity in the tumor section bed, and CCNU given orally. We used a combination of lipid-soluble and -insoluble agents, and also combined a cell cycle-specific agent with a nonspecific agent to enhance the effect of the azaguanine (cell cycle-specific agent). Here survivals seemed again to decrease the effect of radiation therapy in extending survival.

Of the low-grade survival after surgery only, 11 were grade 1 and 1 was grade 2, whereas the group that received radiotherapy contained 18 grade 2's and only 1 grade 1. All the sections were checked by Dr. Valsolomus; Dr. Kenneth Earl of the Armed Forces Institute of Pathology checked those that were in doubt.

The high-grade group again showed a marked increase in survival after combination therapy. Most of our cases survived twice as long as those reported from other centers.

The high-grade group did better when they had surgery and radiotherapy alone. However, when grades 3 and 4 are examined separately, we find that in the surgery and radiotherapy group alone, the 11 grade 3 patients had a mean survival of 402 weeks; this increased the mean survival over the other group, which contained only 8 low- and 22 high-grade gliomas. When we examine these groups separately, we find no significant difference between surgery and radiotherapy, and surgery, radiotherapy, and the combination chemotherapy. The long-term survivals have impressed us; however, we now have 3 long-term survivors in the combination chemotherapy plus radiotherapy group who survived more than 5 years in first-class condition.

L. Marshall: We investigated prospectively the complications of high-dose dexamethasone in the patients Dr. Langfitt described earlier. A group of 121 consecutive neurosurgical patients were started on dexamethasone, randomly allocated to 16, 40, or 80 mg/day and then appropriately tapered. Thirteen of the 27 patients had severe brain-stem signs. Most of the patients had astrocytomas and metastatic carcinomas and were maintained on steroids chronically, i.e., for longer than 30 days. Nineteen members of that group were on steroids for a mean of 206 days.

With respect to complications in these 121 patients, duodenal perforation developed in 1 who had a previous history of ulcer but who was placed on steroids because of intracranial hypertension. Another developed *Candida* esophagitis while receiving radiation therapy, and the third was a postoperative surgical patient.

No complications developed in any of the chronic patients. Five patients developed hypoglycemia and 3 required insulin; 2 of these cleared on steroid withdrawal. We have concluded that in neurosurgical patients, large doses of dexamethasone carry no higher risk than do the low and that chronic administration is not associated with a high incidence of complications. We encountered no complications in these patients and believe that dexamethasone, if it is to be useful, should be used in high doses.

We used methylprednisolone in a pilot study and found that it had an unacceptably high incidence of complications.

One further point: the biology of glioblastoma is such that for at least half the patients, no treatment available in 1976 has any significant effect. To examine the efficacy of what we are doing, we must look beyond that point.

Prognostic Factors for Patients With Brain Tumors[1]

Edmund A. Gehan[2] and Michael D. Walker[3]

ABSTRACT—Patient characteristics of 225 patients entered into a clinical trial conducted by the Brain Tumor Study Group (69-01) were examined for their relationship to survival time. In the clinical study, all patients received surgery and were randomized to control treatment (no further treatment), X-ray therapy (XRT), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), or BCNU + XRT. When treatment was considered as a possible prognostic factor, the patient characteristics most related to survival were: XRT (receiving XRT favorable), age (young, favorable), symptoms of seizures and cranial nerves (both favorable), BCNU treatment (yes, favorable), encapsulated tumor (yes, favorable), and parietal tumor (no, favorable). When treatment was not considered a possible prognostic factor, the patient characteristics most related to survival were: age (young, favorable), biopsy (operation biopsy only, unfavorable), seizures (yes, favorable), cranial nerve symptoms (yes, favorable), and parietal location of tumor (yes, unfavorable). Knowledge of prognostic factors can be used for stratifying patients in clinical studies, for testing the comparability of groups of patients, and in the analysis of the results of clinical studies.—Natl Cancer Inst Monogr 46: 189–195, 1977.

Knowledge of prognostic factors for patients with brain tumors can be used in the planning and analysis of clinical studies; also such information would be useful to patients and family members. In planning clinical studies, patients could be stratified into groups according to prognostic factors and the choice of treatments might be influenced by knowledge of these factors. In the analysis of studies, groups of patients could be tested for comparability through this knowledge, and new treatments might be evaluated by a comparison of observed survival times to those predicted based on knowledge of the prognostic factors; methodology for identifying and using them in clinical studies has been outlined (*1*).

In a clinical trial of surgery plus either mithramycin or control treatment by the Brain Tumor Study Group [BTSG; (*2*)], age and duration of symptoms were identified as two important prognostic indicators. Survival time decreased with increasing age and rose with duration of symptoms. Other factors that were suggested from the previous study were pathologic type [ependymal spongioblastoma patients (unfavorable) and malignant astrocytoma patients (favorable)]; location of tumor in the deep basal ganglia (unfavorable); motor symptoms (unfavorable); signs of visual or ocular motor disturbance (favorable); and involvement of cranial nerves III, IV, and VI (favorable).

The major objective of this study was to identify the important prognostic factors in a clinical trial of BCNU and radiotherapy conducted by the BTSG 69-01. Of the 303 patients entered, all received surgery and were then randomized to receive one of four additional treatments: control (no further treatment), X-ray therapy (XRT), 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), or BCNU + XRT. In this analysis, individual and multiple patient characteristics related to the survival of the patients from time of randomization will be investigated.

MATERIALS AND METHODS

The data for this study are from 303 patients entered in a randomized clinical trial conducted by the BTSG. All patients received surgery and were randomized to receive one of the following: no further treatment, XRT, BCNU, or BCNU + XRT. The patient characteristics considered for their relationship to survival time from randomization were: age, sex, diagnosis, type of operation, location of tumor, symptoms (headaches, personality changes, motor symptoms, seizure, speech, general complaints, sensory, cranial nerves II, III, IV, VI, rapid unconsciousness, cranial nerves, cerebellar, and other), tumor characteristics (hypovascular, invasive, solid, necrotic, soft, friable, cystic, hard, vascular, and encapsulated), and time from symptoms to operation (wk). Data were also available on whether or not a patient 1) had a protocol violation and 2) was in the adequately treated group. There were 225 patients in the Valid Study Group, i.e., patients who were eligible for study and did not have a protocol violation.

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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The analysis of prognostic factors was done on this group of patients.

To determine individual patient characteristics related to prognosis, we considered each characteristic and divided them into categories; survival time was compared between categories either by a generalized Wilcoxon text for comparing two categories (*3*) or a test for comparing more than two treatment groups (*4*). For example, survival curves for males versus females could be compared to determine whether or not sex was related to survival. A criticism of this method of analysis is that it does not take into account correlations among the individual patient characteristics.

To determine multiple patient characteristics related to survival, we used a step-wise regression procedure in which Cox's model was fitted to the survival data. This model is as follows:

$$\lambda(t) = \exp\{\beta_1(X_1 - \bar{x}_1) + \ldots + \beta_k(X_k - \bar{x}_k)\}\ \lambda_o(t)$$

where $\lambda(t)$ is the hazard function at time t, the β's are regression parameters fitted in the model, the X's are patient characteristics possibly related to prognosis, the $\bar{x}$'s are average values of patient characteristics, and $\lambda_{\hat{o}}(t)$ is an arbitrary hazard function when no patient characteristics are included in the model. A hazard function gives the probability of nearly immediate death for an individual known to be alive at time t. A high value of hazard function means that the expected survival time is short, whereas a lower value means a longer survival. The step-wise procedure of fitting the model was as follows: first, the single patient characteristic giving the best prediction of hazard function (or equivalently, survivorship function) was chosen; second, a patient characteristic was chosen so that when it was added to the first, it gave the best pair of patient characteristics; etc. The statistical significance of adding patient characteristics at each stage could be calculated. Methodology for fitting this model is outlined in (*5*).

RESULTS

This section examines the individual and multiple patient characteristics related to survival for the 225 patients in the Valid Study Group.

Individual Patient Characteristics

The median survival time for the 225 patients in the Valid Study Group was 26 weeks. Table 1 gives percentiles of survival time for the various individual patient characteristics considered, and the last column in the table gives the statistical significance of the difference between survival curves for each patient characteristic.

From examining table 1, one can see the following patient characteristics were strongly related ($P = 0.05$ or less) to survival time: age, hypovascular tumors, type of operation, and seizures. Age was related significantly to survival time: the older the age group, the shorter the survival from randomization. Patients with hypovascular tumors had a 40-week median survival time compared with 25 weeks for patients without such tumors. Those with seizures as a symptom had a median survival time of 31 weeks. Patients having a biopsy only had a much shorter median survival time than patients having a subtotal or total resection, i.e., 14 versus 27 weeks ($P = 0.01$).

A moderate relationship (significance level between 5% and about 15%) was noted between certain tumor locations and characteristics and survival time. Patients with occipital tumors lived longer, whereas those with parietal tumors had short survival. The few patients who had tumors in the basal ganglia (BG), thalamus or cerebellum had short median survival times.

A tumor characteristic moderately associated with longer survival time was friability. Patients with hard tumors had short median survival times.

Contrary to what was found in the previous study, sex and duration of symptoms were not significantly related to survival time.

Table 2 gives median survival times by treatment and other classifications of patients (protocol violations and adequate treatment group). The difference among treatments was statistically significant ($P<0.001$); the rankings of the treatments according to median survival time were: XRT (median 36 wk), BCNU + XRT (median 34 wk), BCNU (median 18 wk), and control treatment (median 14 wk). The most marked differences in median survival time were between those groups of patients receiving XRT versus patients not receiving irradiation. However, we had evidence that patients given BCNU survived longer than patients receiving control treatment ($P < 0.05$).

We had no statistical evidence that patients having a protocol violation had significantly different survival than those who did not ($P = 0.70$), though the median survival time was 33 weeks for the former group and 26 weeks for the latter. Evidence that patients who were treated adequately had a significantly longer survival time was pronounced; they had a median survival time of 30 weeks compared with 9 weeks for those whose treatment was inadequate ($P<0.01$).

Multiple Patient Characteristics

In this section, combinations of patient characteristics are considered for their relationship to survival. In this approach, we allowed for patient characteristics that are highly correlated, as well as for variables defined as a product of two patient characteristics, such as age × seizures, which are sometimes designated "interaction" effects.

Because of the multiplicity of patient characteristics, the analysis proceeded in several stages. In the first or screening stage, individual patient characteristics with a large number of categories were analyzed separately for relationships to survival time and, in the second stage, patient characteristics chosen from the first stage plus the remaining characteristics were considered for inclusion in the model. In the screening stage, the patient characteristics considered were (number of categories given in parentheses): symptoms (12), tumor characteristics (10), location of tumor (6), type of operation (6), and diagnostic type (3).

For each set of patient characteristics, Cox's model (outlined in the "Methods section") was fitted to the data relating hazard function to patient characteristics determined in order of relative importance. In the analysis of symptoms related to survival, the first three selected in the step-wise fitting procedure were seizures ($P=0.002$; those with seizures having longer survival), motor symptoms ($P=0.039$; those with motor symptoms having shorter survival), and cranial nerves ($P=0.146$; those with symptoms in the cranial nerves having longer survival). In this screening stage, those characteristics that were statistically significant at the 0.05 level or marginally significant (such as $P=0.146$) were passed to the next stage.

Among the 10 tumor characteristics, those in order of inclusion in the model were hypovascular and encapsulated tumors ($P=0.039$ and $P=0.076$, respectively; those with such tumors having longer survival). No other characteristics were near statistical significance at a $P=0.10$ level.

Among the six tumor sites, two were statistically significant: BG thalamus and parietal tumors ($P=0.004$ and $P=0.004$, respectively, those with such tumors having significantly shorter survival).

Among the types of operations, patients having a biopsy only had significantly shorter survival than all others ($P<0.001$). When classified by pathologic type, patients with glioblastoma multiforme or malignant glioma had significantly shorter survival than those with other diagnoses ($P<0.001$).

The patient characteristics (for inclusion in the survival model in the next stage) were considered including and excluding treatment as a possible patient characteristic related to survival (table 3).

Table 4 gives the patient characteristics in order of relative importance for predicting survival time when treatment was included. The *P*-value indicates that the first six patient characteristics were statistically significantly related to survival time ($P < 0.05$), and the sign of the regression coefficient indicates the nature of the relationship, a positive sign indicating a higher hazard function (or a shorter survival) and a negative sign indicating a lower hazard function (or a longer survival). In order of relative importance in predicting survival time, the following characteristics were included in the regression model: XRT (yes, favorable), age (young, favorable), seizure × cranial nerves (yes on both, favorable), BCNU (yes, favorable), encapsulated (yes, favorable), and parietal (no, favorable). On the right-hand side of table 4, relative risk values are given when a particular characteristic was at a favorable or an unfavorable rating. If one considers the overall survival experience of the group of 225 patients as unity, then subgroups of patients can be compared in relative risk with the overall group. For example, in table 4, if one does not receive XRT, the risk of death per unit time is 1.9 × the average. If one does receive radiotherapy, the risk is .7 × the average. The last column in the table gives the ratio of relative risks for favorable to unfavorable cases. For XRT, the ratio is .37, indicating that the risk of death per unit time for a person who is receiving radiotherapy is 37% of that for a patient who is not. Ratios of relative risks that were under .50 were: XRT, age, and seizure × cranial nerves. The interpretation of the significance of the product variable is as follows: Having neither seizures nor cranial nerves as a symptom indicates an unfavorable relationship to survival time; having both symptoms indicates a favorable relationship; and having either symptom is also favorable, but by a lesser amount than when both are present. Cox's model for these data is given at the bottom of table 4.

Text-figure 1 gives survival curves for patients grouped according to the predicted value of the individual median survival time. The patients were grouped arbitrarily into four intervals, and it may be noted from the text-figure that there is a clear separation among the survival curves; the differences among all pairs of curves are statistically significant at a $P<0.05$ level. We found close

TABLE 1.—*Percentiles of survival times (wk) by patient characteristics (Valid Study Group)*

Patient characteristic	No. patients	No. deaths	Percentiles of survival time, wk			*P*
			75	50	25	
Age, yr						
0–49	61	54	21	40	60	0.005
50–59	76	75	16	29	49	
60 and over	88	86	10	18	29	
Diagnosis						
Glioblastoma multiforme and malignant glioma	191	184	12	25	43	0.19
Malignant astrocytoma	18	16	12	33	53	
All others	16	15	17	36	63	
Sex						
Male	143	135	14	27	49	
Female	82	80	11	25	40	0.24
Location						
Cerebellum	3	3	3	18	43	0.15
BG thalamus	2	2	7	14	16	
Frontal	72	67	11	25	49	
Occipital	15	14	23	43	57	
Parietal	59	59	10	21	34	
Temporal	74	70	17	27	47	
Tumor characteristics						
Hypovascular						
No	207	199	12	25	44	0.03
Yes	18	16	17	40	72	
Invasive						
No	98	9	14	25	47	0.78
Yes	127	12	12	27	47	
Solid						
No	161	156	14	27	48	0.44
Yes	64	59	12	22	42	
Necrotic						
No	110	102	13	24	47	0.95
Yes	115	113	13	27	47	
Soft						
No	115	111	12	24	44	0.33
Yes	110	104	14	28	48	
Friable						
No	195	187	12	24	46	0.055
Yes	30	28	15	35	65	
Cystic						
No	174	165	13	27	49	0.21
Yes	51	50	11	25	33	
Hard						
No	200	191	14	28	48	0.13
Yes	25	24	10	14	41	
Vascular						
No	153	147	12	25	45	0.45
Yes	72	68	14	28	48	
Encapsulated						
No	212	203	12	26	47	0.28
Yes	13	12	15	26	64	
Symptoms						
Headache						
No	97	94	14	28	47	0.56
Yes	128	121	12	25	48	
Personality change						
No	144	137	12	27	48	0.85
Yes	81	78	14	25	46	
Motor symptoms						
No	111	105	14	27	50	0.37
Yes	114	110	12	26	43	
Seizure						
No	163	160	13	24	42	0.05
Yes	62	55	13	31	58	
Speech						
No	166	157	14	27	48	0.43
Yes	59	58	10	25	43	

TABLE 1 (continued)

Patient characteristic	No. patients	No. deaths	Percentiles of survival time, wk			P
			75	50	25	
General complaints						
No	209	199	13	26	48	0.68
Yes	16	16	20	27	40	
Sensory						
No	194	186	13	27	47	0.70
Yes	31	29	12	23	42	
Cranial nerves II, III, IV, VI						
No	208	198	13	25	46	0.13
Yes	17	17	16	34	68	
Rapid unconsciousness						
No	212	202	13	26	48	0.82
Yes	13	13	16	26	46	
Cranial nerves other						
No	217	207	13	26	48	0.62
Yes	8	8	21	27	33	
Cerebellar						
No	222	212	13	27	47	0.77
Yes	3	3	7	12	29	
Other						
No	182	173	14	27	48	0.32
Yes	43	42	10	21	43	
Time from symptoms to operation, wk						
0–25	200	191	12	27	47	0.85
26–51	12	12	13	19	71	
52 or more	13	12	15	22	38	
Left or right						
1) Left	102	99	11	22	46	0.13
2) Right	123	116	16	28	47	
Type of operation						
Biopsy only	12	12	9	14	19	0.01
Partial or total resection	213	203	14	27	48	
All patients	225	215	13	26	48	

accord between observed and predicted median survival times, which indicated that the prognostic factors included in the model predict survival well.

When treatment was not considered as a possible patient characteristic, the step-wise regression analysis showed the following characteristics to be related significantly to survival (table 5): age, operation biopsy only, symptoms of seizures and cranial nerves, and parietal location of tumor. Favorable patient characteristics were: young age group, operation not biopsy only, having seizures and cranial nerves as symptoms, and location of tumor at a site other than parietal. From consideration of the relative risks, the patient characteristics with the strongest relationship to survival time were: age of the patient and a biopsy only; for both characteristics, the ratio of relative risks was .51 or less.

Text-figure 2 depicts the survival curves according to predicted median survival times for each patient, with the equation given at the bottom of table 5. There is a clear separation in the survival experience of 3 of the 4 groups of patients, and the observed median survival times are in reasonably close accord with those predicted. Hence the equation could be used to classify patients differentially according to expected survival experience.

DISCUSSION

If the patient characteristics with the strongest relationship to survival time are designated to be those identified when treatment was included or excluded, then the most important characteristics are: age, symptoms of seizures and cranial nerves, and parietal location of tumor. Age of the patient was identified previously as important in the mithramycin study (*2*), and the presence of cranial nerve symptoms was previously of suggestive significance. Having seizures as a symptom or parietal location of tumor was not identified previously as being important. We hope to examine these characteristics in future studies to determine the consistency of the relationship.

TABLE 2.—*Percentiles of survival time by treatment and other classifications of patients in the Valid Study Group*

Patient characteristic	No. of patients	No. of deaths	Percentiles of survival time, wk			
			75	50	25	*P*
Treatment						
Control	31	31	9	14	21	<0.001
XRT	70	65	18	36	50	
BCNU	51	51	9	18	28	
BCNU+XRT	73	68	16	34	60	
Adequate treatment						
No	58	56	4	9	26	=0.002
Yes	167	159	18	30	49	
Total	225	215	13	26	48	
Protocol violations	78	72	9	33	49	=0.70

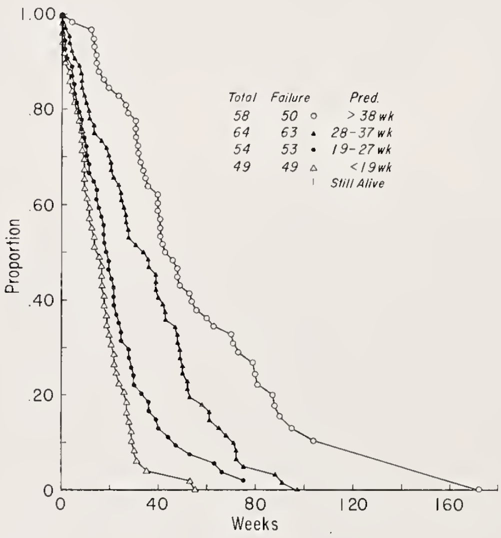
TABLE 3.—*Codes for treatment and patient characteristics studied for relation to survival time*

Characteristic	Codes
XRT	0=none, 1=alone or with BCNU
BCNU	0=none, 1=alone or with XRT
Age	0=less than 50, 1=50–59, 2=60+
Symptoms to operation	No. of wk
Diagnosis	2=glioblastoma multiforme + malignant glioma, 1=other
Operation biopsy only	1=no, 2=yes
Tumor characteristics	
Encapsulated	1=no, 2=yes
Hypovascular	1=no, 2=yes
Tumor location	
BG thalamus	0=no, 1=yes
Parietal	1=no, 2=yes
Seizures	1=no, 2=yes
Cranial nerve symptoms	1=no, 2=yes
Motor symptoms	1=no, 2=yes

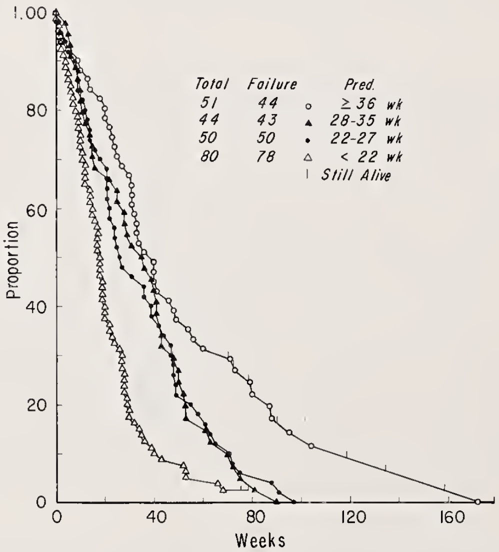
In contrast to the mithramycin study in which duration of symptoms was extremely important, no significant relationship to survival time could be demonstrated.

The regression models at the bottom of tables 4 and 5 can be used for several purposes in future studies. First, patients entering study might be stratified into groups according to predicted median survival time, and randomization among different treatments could be made within each prognostic category. Second, average predicted median survival times could be calculated for patients on each of the treatments to determine whether there were any real differences among groups with respect to the major prognostic factors. Third, the regression model at the bottom of table 4 could be used to predict median survival times, assuming the patients were to receive XRT, BCNU, or both. If some other combination treatment is being evaluated, an approach to the analysis would be to compare observed survival times on the new treatment to those predicted following BCNU, XRT, or both.

The regression model may be used to calculate average predicted median survival times for patients in each treatment group of the BCNU + XRT study (BTSG 69-01). The observed and predicted median survival times in weeks for each treatment are as follows: no treatment (14, 26); XRT (36, 32); BCNU (18, 26); BCNU + XRT



TEXT-FIGURE 1.—Survival curves for 4 groups of patients by predicted median survival time-treatment included.



TEXT-FIGURE 2.—Survival curves for 4 groups of patients by predicted median survival time-treatment excluded.

TABLE 4.—*Patient characteristics related to survival in order of relative importance in survival model (treatment included)*

Patient characteristic	Regression coefficient	*P*	Relative risk: Favorable[a]	Relative risk: Unfavorable[a]	Relative risk: Favorable/unfavorable ratio
XRT	−1.0011	<0.001	.70	1.90	.37
Age	+0.3649	<0.001	.66	1.38	.48
Seizure × cranial nerve	−0.4416	0.008	.32	1.19	.27
BCNU	−0.4040	0.008	.83	1.25	.67
Encapsulated	−0.6923	0.011	.52	1.04	.50
Parietal	+0.3636	0.027	.91	1.31	.70

Cox's regression model relating hazard function to patient characteristics (treatment included)

$$\ln\left(\frac{\lambda(t)}{\lambda_0(t)}\right) = -1.0011\,(\text{XRT}-.64)+.3649\,(\text{age}-1.12) -.4416\,(\text{seizure}\times\text{cranial nerve}, -1.40) -.4040\,(\text{BCNU}-.55)-0.6923\,(\text{encapsulated}-1.06) +.3636\,(\text{parietal}-1.26).$$

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[a] Codes for favorable, unfavorable characteristics: XRT (1, 0), age (0, 2), seizure × cranial nerve (4, 1), BCNU (1, 0), encapsulated (2, 1), parietal (1, 2).

(34, 28). Patients receiving XRT had the most favorable predicted median of 32 weeks, and the other groups did not differ substantially from one another. The effect of each treatment may be estimated by taking observed minus predicted times: no treatment (−12 wk); XRT (4 wk); BCNU (−8 wk); and BCNU + XRT (6 wk). The estimated average benefit in median survival time for patients receiving XRT versus no XRT was 14.5 weeks; and for patients receiving BCNU versus no BCNU was 3.5 weeks. Patients receiving BCNU + XRT would have an estimated median survival time 2 weeks longer than patients receiving XRT alone.

TABLE 5.—*Patient characteristics related to survival in order of relative importance in survival mode (treatment excluded)*

Patient characteristic	Regression coefficient	*P*	Relative risk: Favorable[a]	Relative risk: Unfavorable[a]	Relative risk: Favorable/unfavorable ratio
Age	+0.3339	<0.001	.69	1.34	.51
Biopsy	+0.8086	0.009	.96	2.16	.44
Seizure	−0.4407	0.027	.73	1.13	.65
Cranial nerves	−0.5519	0.017	.61	1.06	.58
Parietal	+0.3243	0.046	.92	1.27	.72

Cox's regression model relating hazard function to patient characteristics (treatment excluded)

$$\ln = \left(\frac{\lambda(t)}{\lambda_0(t)}\right) = 0.3339\,(\text{age}-1.12)+.8086\,(\text{biopsy}-1.05) - 0.4407\,(\text{seizures}-1.28)-.5519\,(\text{cranial nerve}, -1.11)+0.3243\,(\text{parietal}-1.26)$$

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[a] Codes for favorable, unfavorable characteristics: age (0, 2), biopsy (1, 2), seizures (2, 7), cranial nerves (2, 1), parietal (1, 2).

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Brain Tumors: Criteria of Response and Definition of Recurrence[1,2]

Charles B. Wilson,[3] David Crafts,[4] *and* Victor Levin[5]

ABSTRACT—The criteria for determining response to therapy and establishing brain tumor recurrence are presented. Basing their conclusions on a series of more than 500 patients, the authors contend that serial scintiscans and computerized axial tomograms can be used to determine accurately the response to therapy and recurrence or tumor regrowth. The problems in evaluating response and tumor recurrence are discussed, with emphasis on those relating to concurrent glucocorticoid therapy and medical conditions such as infection, hematomas, and drug side-effects.—Natl Cancer Inst Monogr 46: 197–203, 1977.

In the present context, the terms "response" and "recurrence" will refer to recognizable changes in the bulk of an intracranial mass composed of proliferating and nonproliferating tumor cells, nonviable tumor cells and debris, extracellular fluid, hematoma, and supporting nonneoplastic stroma.

Although the ideal indicator of a tumor's activity would be a quantitative measurement of clonogenic tumor cells, this is only a faint hope for the foreseeable future. Our present methods for determining either a decrease or an increase in tumor mass are relatively crude; first, they are insensitive to changes smaller than 0.2–0.3 of the tumor mass; and second, present techniques are rarely useful in defining a mass containing 10^{10} or fewer cells. Translated into practice, this means that the present methods can be applied only to tumors with a bulk of 10 g or greater, and that they will not detect changes of less than 25%.

Certain small tumors (e.g., in the brain stem) produce devastating deficits, and their local infiltrations may escape detection by radionuclide and computerized axial tomographic (CAT) scans, the two procedures currently used to follow the course of an intracranial tumor. In this situation, response or recurrence can be determined best by meticulous serial neurologic examinations.

Finally, if neural structures, including gray and white matter, have been irreversibly damaged, a therapeutically induced reduction in tumor mass may not be accompanied by improved neurologic function. Although we have observed few instances of this kind, *sole* reliance on the neurologic examination may lead the physician to misinterpret the tumor's behavior.

Viewed simply, response and recurrence represent extreme poles of the actual situation: Response necessarily expresses a therapeutic reduction in tumor mass, whereas recurrence defines a tumor's progression either with or without concurrent treatment. At present, the tools by which we make these judgments are the same: clinical neurologic examination, radionuclide and CAT scans, electroencephalogram (EEG), special neuroradiologic procedures (angiography, pneumography, and myelography), and examination of the cerebrospinal fluid (CSF). Response is less difficult to define than recurrence, although both have pitfalls. In the following sections, we will define response and recurrence on the basis of our experience with 500 patients observed from the beginning of treatment to death. Text-figure 1 indicates the flow of patients within the chemotherapy service and their routing for treatment.

RESPONSE IN RELATION TO DRUG PROTOCOL

Our primary concern in this paper is response in traditional phase II trials, but for clarification a distinction should be made between the different requirements for entry and end points of phase II and phase III trials.

The phase II (and phase I–II) trial is designed

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

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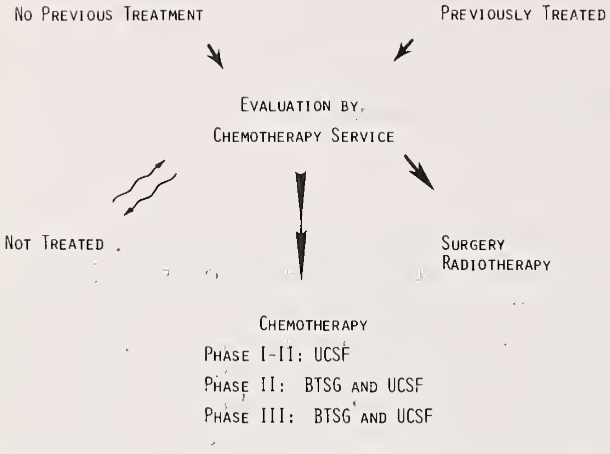

TEXT-FIGURE 1.—Flow chart indicating procession of patients on chemotherapy protocols of the Brain Tumor Study Group (BTSG) and the Neurosurgical Department's Clinical Cancer Chemotherapy Service.

to identify therapeutic activity, and for this purpose any patient bearing an expanding tumor is a suitable subject. (Entry requirements will be considered in the following section.) In this type of trial, the investigator wants to determine the frequency of therapeutic responsiveness; here the duration of response assumes secondary importance. A phase II trial may accrue patients in either a sequential or a randomized manner and, in the latter instance, patients are randomized into two or more phase II protocols.

The phase III trial, in which a comparison of a new form of treatment with a standard or best-known therapy is made, is prospective and randomized into two or more arms. The patients must constitute a homogeneous population in respect to tumor type, preliminary treatment, ancillary therapy, e.g., steroids and continuing care. The BTSG has defined the phase III entry requirements for patients harboring anaplastic gliomas, and current Group protocols reflect the experience gained in 3 earlier studies. At the moment, death is the only valid end point of a phase III trial, but considerable effort is being expended to define recurrence with sufficient accuracy to permit this event to be used as an end point. In addition to moral questions, this would give the evident advantages of an earlier "off-study" time and the potential for crossover studies.

PHASE II REQUIREMENTS FOR ENTRY

Table 1 lists the requirements for entry into our phase II trials; most of the patients who enter have recurrent tumors. Also eligible for phase II trials are patients who, on the basis of clinical evidence short of operative confirmation, harbor either primary or metastatic brain tumors. Neuroradiologic procedures must provide *unequivocal* evidence of a mass, the history and neurologic examination must confirm the presence of a tumor, and all available evidence must support no alternative diagnosis. Primary tumors in the histologically unverified category will occupy three sites: brain stem, deep midline (basal ganglia and corpus callosum), and motor cortex in the dominant hemisphere. In our series of patients treated without histologic diagnosis, postmortem examination has never failed to verify the presence of a tumor. The presumptive diagnosis of metastatic brain involvement is based on the presence of one or more intracranial tumors in a patient with a known primary source outside the central nervous system (CNS). Leptomeningeal carcinomatosis presents a special problem, and because the condition is both uncommon and often unaccompanied by measurable tumor mass, it will be excluded from further discussion.

Deterioration beginning more than 2 months after operation constitutes presumptive evidence of tumor regrowth if complications, e.g., hydrocephalus, are excluded. Reasons for the lapse of 3 months after completion of radiotherapy are discussed in the section on recurrence. Because response to a chemotherapeutic agent may be delayed, the interval of 6 weeks following administration of a drug (table 1, #5) should be observed. For the nitrosoureas, 8 weeks should elapse before therapy with a new agent is initiated, in view of the delayed response and myelotoxicity observed in patients receiving either 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) or 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU).

Approximately one-third of the patients referred as candidates for phase II trials are not treated for the reasons indicated in table 2.

EVALUATION POTENTIAL AND RESPONSE IN PHASE II TRIALS

Patients for evaluation are those who fulfill all requirements for entry, complete at least 1 full course of therapy, and live for at least 2 months after initiation of treatment.

In table 3, the procedures are indicated that constitute the basis for entry into a phase II trial and also provide a base line for later comparison in evaluating response to treatment. The procedures that are repeated at the time of each reevaluation and re-treatment are cited in the footnotes. Certain of these, e.g., myelography

TABLE 1.—*Phase II entry requirements*

1) Unequivocal neurologic deterioration secondary to tumor growth and a tumor that is demonstrable by neurodiagnostic procedures
2) A pathologic diagnosis or a clinical picture supporting no alternative diagnosis
3) At least 2 mo after preceding surgery
4) At least 3 mo after the completion of radiotherapy
5) At least 6 wk (8 wk for nitrosourea) after preceding chemotherapy
6) Life expectancy of 2 mo or longer

TABLE 2.—*Reasons for not treating patients evaluated for phase II trials*

1) No evidence of tumor regrowth
2) Insufficient interval after earlier treatment (operation, radiotherapy, and chemotherapy)
3) Operation recommended (removal of tumor or shunt)
4) Radiotherapy recommended
5) Deterioration due to cause other than tumor (mental depression, radiation necrosis, cerebrovascular disease, benign arachnoid cyst)
6) Complicating factors (wound infection, CSF fistula)
7) Advanced neurologic deficit precluding reasonable hope of worthwhile recovery (investigator's decision)
8) Life expectancy less than 2 mo
9) Treatment declined by patient (or family)

TABLE 3.—*Procedures used for determining entry and response*

1) Neurologic examination[a]
2) Radionuclide scintiscan[a]
3) CAT scan[a]
4) EEG[a,b]
5) Neuroradiologic procedures[c] (angiography and pneumography)
6) Examination of CSF[d] (cytology, polyamines)
7) Myelography[e]

[a] Procedures are repeated at the time of each reevaluation and re-treatment.

[b] This test has limited value.

[c] These procedures are rarely repeated.

[d] CSF examinations are used selectively.

[e] This is seldom used except in patients who have medulloblastoma with suspected or known spinal implants.

and CSF cytology, are applied selectively under special circumstances. Although the work is still in an investigative stage, analysis of CSF for polyamines has provided data that show that these substances are reliable though nonspecific indicators of replicating cell mass and tumor cell-kill. The CAT scan is a new means of visualizing the brain and its contents but at present, we use it to complement the radionuclide scan. We are in the process of combining the information obtained from all procedures performed serially (cited in first footnote of table 3) to determine the value of each procedure, individually and in combination with other procedures, as a predictor of response and recurrence (*1*).

Undoubtedly, the most valuable procedure is the clinical neurologic examination. When possible, serial examinations should be performed by the same examiner; if not, unambiguous recording of neurologic function permits valid comparisons over the course of time. Neurologic examination includes recording of visual fields and psychologic testing when indicated.

Early experience with serial cerebral angiography revealed its limited value as an indicator of tumor regression (*2*), and because of morbidity and expense, we discontinued its use.

In determining the oncolytic effectiveness of an agent, we assign first importance to the neurologic examination. The correlation between clinical improvement and an improved scan (and the converse) is good but imperfect (*3*). We use the scan as a second indicator of drug effect for the following reasons: 1) An improved scan may precede neurologic improvement, and vice versa; 2) in neurologically "silent" areas, e.g., frontal pole, neurologic examination may provide little clinical indication of a change in tumor mass; 3) certain neurologic deficits, e.g., hemianopia, represent irreversible damage, and unequivocal tumor regression may be accompanied, although rarely, by no improvement in neurologic function; and 4) clinical and pathologic experience have established the reliability of sequential brain scans in following the course of intracranial tumors.

Patients for evaluation are assigned to one of three categories of response based on a review of 2 or more recorded neurologic examinations and corresponding brain scans:

Responder.—Unequivocal clinical neurologic improvement and brain scans demonstrating a smaller area of abnormal permeability, i.e., mass; for multiple metastatic tumors, unequivocal shrinkage of 1 tumor and stability of other tumor(s) is acceptable. In addition, these changes must be observed while the patient is on nonescalating doses of dexamethasone.

Probable responder (*stable disease*).—Either one of two responses qualifies for this category: 1) improvement in clinical status and an unchanged brain scan or an improved brain scan and a stable clinical course; 2) arrest of a deteriorating course, with neither progression nor improvement in neurologic condition and brain scan, with stability for a period of 3 months or longer in a patient harboring a malignant tumor (glioblastoma, malignant astrocytoma, medulloblastoma, and cerebral metastasis).

Nonresponder.—Unimproved or worsened clinical condition and brain scan.

Steroids.—We use dexamethasone to combat neurologic deterioration when a patient's functional capacity is severely compromised. Many patients are receiving dexamethasone (or a similar steroid) when initially referred for treatment. In this group, the minimal maintenance dose required to achieve the desired clinical state must be established, and when this dose is maintained for 48 hours or longer, a new base-line, neurologic examination and brain scan should be obtained. If treatment with glucocorticoids is initiated at the time of initial evaluation, the same procedure is followed in establishing, by increments, a minimal maintenance steroid requirement.

Although glucocorticoids inhibit the growth of a murine glioma (*4*), we have assumed that they possess no antitumor activity in man, and clinical improvement following their use has been ascribed to recognizable, though poorly explained, anti-edema properties. In earlier reviews, we accepted a significant reduction (or elimination) of maintenance steroid requirement as one indication of tumor regression. Although this judgment has probable validity, we do not use it as a criterion of responsiveness; however, in every patient designated as a responder, we have been able to either reduce or eliminate maintenance glucocorticoid requirements. With rare exceptions, steroid maintenance can be reduced or stopped in patients with responsive tumors, and before administering each subsequent course to responding patients, the investigator should attempt to reduce steroid maintenance doses.

The criteria for determining actual and probable response are summarized in table 4. We have combined responders and probable responders in arriving at an overall response rate, using the total number of patients who could be evaluated as the denominator.

TABLE 4.—*Criteria of response (phase II)*

Unequivocal response
  1) Unequivocal improvement in neurologic function
  2) Unequivocal improvement in radionuclide scan
  3) Nonescalating maintenance dose of glucocorticoid

Probable response (stable disease)
  1) Improvement in either neurologic function or radionuclide scan without deterioration in the other *or* stability of neurologic function and radionuclide scan for 3 mo (malignant tumors)
  2) Nonescalating maintenance dose of glucocorticoid

RECURRENCE: DEFINITION AND CRITERIA

The time of recurrence indicates that point when regrowth of a previously treated tumor is established by presently available techniques. Unequivocal evidence of recurrence is used in two ways: First, recurrence is one requirement for entry into a phase II trial and, in this situation, recurrence is established by a history of deteriorating neurologic function and the demonstration of a mass with the characteristics of a recurrent tumor; second, the time of first recurrence is a highly desirable, but so far unattained, end point for phase II and III trials in which serial examinations should provide an ideal basis for making this determination. Requirements for patients entering phase II trials with recurrent tumors have been discussed, and the remainder of this section will be restricted to the early detection of tumor regrowth or progression during or after a course of therapy, whether the therapy utilizes single agents or multiple modalities.

The diagnosis of recurrence is made difficult because of the anatomic and physiologic distortions produced by earlier treatment and residual neurologic deficits. The standard diagnostic tests are less reliable since abnormality must now be relative to previous abnormal studies. Loss of brain substance, scarring, and, in some cases, residual but not actively regrowing tumor may obscure the clinical manifestations of tumor regrowth.

In establishing tumor recurrence in the patient under continuing observation, serial examinations provide the basis for defining two points: "recurrence suspected" and "recurrence confirmed." In most patients, recurrence will be suspected from a few to several weeks before it becomes obvious, but the interval is variable. In at least one-third of the patients suspected of recurrence, subsequent events indicate that the suspicion was a false alarm. This has been true whether the suspicion was based on subtle neurologic symptoms and signs or on minor changes in radionuclide and CAT scans. The reasons for the misleading indications of early recurrence relate to well-known variability in the functional capacity of patients with CNS disease: in the case of misleading scans, the explanation may be observer error, technical error in the timing of the study, or differences in CAT equipment and positioning of the patient. Consequently, "recurrence suspected" is not a reliable end point, although it serves to alert the investigator.

Recurrence confirmed indicates unequivocal evidence of regrowth; because it can and should provide a clear end point in phase III studies, the criteria must be strict. Proposed criteria for recurrence are indicated in table 5. Whereas we are unwilling to use the CAT scan to define response, we consider an unequivocal change in serial CAT scans a reliable indication of regrowth.

TABLE 5.—*Criteria for confirmed recurrence*

1) Deterioration in neurologic function and worsening of either radionuclide or CAT scan[a]
2) Unequivocal evidence of distal tumor implantation, intracranial or intraspinal

[a] If both scans remained normal or are uninterpretable because of artifact, angiography and pneumography should be performed but with the disadvantage of lacking a preceding study for comparison.

Distal implants are uncommon with tumors other than medulloblastomas and anaplastic ependymomas, but their appearance at any time after initial radiotherapy indicates treatment failure tantamount to recurrence for our purposes.

Many symptoms such as headache and lethargy are nonspecific and unreliable as indicators of recurrence. Evidence of increased intracranial pressure without accompanying deterioration in another sphere is also an unreliable indication of regrowth. The same is true of CSF cytology and the EEG. Additional experience may modify our present position in defining recurrence, but the proposed criteria will avoid mistaken diagnosis. Earlier detection of recurrence is a desirable goal, but present methods and limited experience in discovering the first evidence of regrowth require that we adhere to exacting criteria to avoid the pitfalls described in the following section on differential diagnosis.

RECURRENCE: DIFFERENTIAL DIAGNOSIS

In the immediate postoperative period, deterioration raises the surgical problem of cerebral edema or hematoma versus continued growth of inadequately decompressed tumor. Cerebral edema reaches its peak in the first 2–4 days after surgery, and hematomas usually become evident within the first few days after operation. As a rule, improvement following the administration of steroids supports the initial diagnosis of edema, although steroids also benefit the postoperative hematoma. Postoperative impairment of CSF flow or the tumor itself can cause hydrocephalus, which may appear several weeks after surgery or much later. Drowsiness and decreased mentation may develop in the absence of any new abnormality seen on the brain scan. For all the preceding diagnostic possibilities, the CAT scan provides a noninvasive means for identifying the problem.

Postoperative radiotherapy can create diagnostic confusion in four ways:

1) During the course of radiation, there may be reactive edema with nausea, drowsiness, and worsening of deficit, especially in the poorly decompressed tumor. Ordinarily, this is not a serious diagnostic problem and resolves with temporary increase in steroids and a lapse of perhaps several days without further radiotherapy.

2) A more difficult and less well-known complication is created by transient encephalopathy that may come on 1–15 weeks (usually 6–10) after the end of X-ray therapy (*5*). It can develop after exposure of as little as 2,400 rads in children who are receiving CNS prophylaxis for leukemia (*6*). The syndrome is usually manifested by drowsiness, nausea, and malaise, but can include ataxia, dysphasia, and exaggeration or reappearance of previous neurologic deficits. It does not require a preexisting brain lesion and probably is related to demyelination, produced either by direct effect on oligodendroglia or by provoking an autoimmune reaction (*7*, *8*). The tissue is edematous, friable, and vascular, and surgery is not helpful (*9*). Steroids are effective, but the only way to differentiate this condition from tumor recurrence is to suspect it because of the timing and to temporize by providing symptomatic care. The patient with radiation encephalopathy will improve without specific treatment, usually within 4–6 weeks. The encephalopathy is ordinarily self-limited and completely reversible (although 1 patient of Lampert et al. (*7*) died with patchy areas of demyelination disseminated in the irradiated area of brain). The patient may then do well without steroids for many months or years before actual tumor regrowth.

3) Better known is the condition of radiation necrosis, although it is less frequent now due to the standardization of doses and improving dosimetry. This condition may develop as early as 4 months or as late as 9 years after irradiation, though it occurs most often after 1–2 years (*10*). The symptoms may mimic tumor regrowth (*11*). Usually, late studies will show atrophy (*12*), but coagulative necrosis can create a mass (*13*) that, even on gross tissue examination, resembles a tumor (*14*). Scan and angiogram may show a mass but not neovascularity. The diagnosis may be suspected from the history of radiotherapy; however, surgery is required sometimes both for definitive diagnosis and to remove the mass. Steroids may help transiently but the outcome is often fatal.

4) Necrosis of tumor after X-ray can leave a pseudocyst, which can cause symptoms by mass effect (*10*).

The late onset of seizures or the aggravation of preexisting seizures may or may not be the consequence of tumor regrowth. Although seizures, viewed as a symptom, can first appear or increase in severity and frequency with tumor recurrence, with equal probability the underlying cause is an epileptogenic focus independent of recurrent tumor. Seizures can produce neurologic symptoms suggestive of recurrence, especially in the postictal state. Ordinarily, postconvulsive deficits disappear within a few hours, but they may persist for several days. Uncommonly, deficits may be caused by ongoing seizure without readily recognized signs of seizure (subclinical status epilepticus). EEG establishes the diagnosis, and neurologic improvement will follow seizure control. The possibility that an unexpected seizure heralds tumor regrowth requires appropriate investigation and treatment.

Cerebral infarction can be spontaneous and unrelated (from arteriosclerosis), can occur as a consequence of postoperative impairment of circulation (for example, surgical occlusion or narrowing of major arteries), or can be a direct result of tumor growth, with constriction or frank invasion of arteries and more rarely of veins. An occasional patient presents with a suddenly increased deficit, an avascular mass near the previous tumor site, a positive scan focus, and stenosis or occlusion of a major artery in the area. The symptoms may or may not be related to arterial occlusion, and if they are, the occlusion may or may not be caused by tumor growth. Usually, no postoperative angiogram is available for comparison, and the decision for treatment may have to be an educated guess, taking into account factors such as the patient's age and any history of gradual deterioration prior to the principal episode. In most malignant gliomas, the late appearance of a strokelike syndrome is associated with tumor regrowth.

Similarly, a cerebral hemorrhage may be spontaneous and anatomically unrelated to the tumor site. More often, spontaneous bleeding occurs within the tumor, occasionally during regrowth but frequently as a complication of effective chemotherapy. If suddenness of onset, angiogram, and CAT scan support the diagnosis of hematoma, then treatment, whether surgical or conservative, is directed initially to the hematoma.

Metabolic and other generalized systemic problems also can cause neurologic disorders, including focal deficits, if there is underlying brain damage. Hypoglycemia, acidosis, hypotension, uremia, hepatic failure, anemia, electrolyte imbalance, respiratory insufficiency, fever, and infection can increase focal deficit with or without alteration in consciousness. It is sometimes surprising how much worse a patient with severe deficit can appear even a week after a urinary tract infection. These conditions can be eliminated during the course of a thorough evaluation.

Depressant drugs, e.g., phenobarbital and primidone, can have similar effects, and other compounds such as diphenylhydantoin may have neurologic side-effects. In this respect, information regarding serum drug levels may be invaluable. If the patient is receiving anticancer chemotherapy, methotrexate administered intrathecally can cause leukoencephalopathy; procarbazine can cause drowsiness, nausea, and vomiting; vincristine is rarely associated with seizures; and nitrogen mustard sometimes causes cerebral edema (*15*). The patient who is deteriorating and is on maintenance doses of steroids must be checked for irregularity of dosage.

Simple fatigue can intensify a deficit, especially relatively sensitive functions such as dysphasia. It is common to have dysphasia improve the morning after a night-time admission to the hospital. Similarly, emotional depression can cause an apparent deterioration, with decreased physical activity and speech, and increased headaches. On the other hand, tumor regrowth can cause such a mood alteration, especially if it involves the frontal or temporal lobes. Understanding the social dynamics of the family can be useful (because they affect the patient's emotional state) and possibly also the history that the physician obtains.

Meningitis or abscess in the tumor bed may cause diagnostic difficulty at any time, though most commonly within the first 2 postoperative weeks. Such rapid onset tends to differentiate infection from tumor regrowth, but lumbar puncture, aspiration, or reexploration may be necessary, depending on clinical findings. An abscess developing later, e.g., related to an open sinus or air cell, or to the presence of foreign bodies, though rare, must be kept in mind, especially if treatment for tumor recurrence will not include more surgery. Angiography, with attention to the pattern of neovascularity and amount of edema, and CAT are helpful in this regard.

Finally, the patient known to harbor a brain tumor may contract an independent neurologic disease. Collagen diseases, degenerative diseases, multiple sclerosis, viral encephalitis (*16*), subdural hematoma, or a different tumor (e.g., meningioma in a patient who has had surgery for astrocytoma) could easily be overlooked. Most of these diseases cause symptoms atypical for recurrent tumor, and they can be correctly identified by thoughtful and careful neurologic testing.

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DISCUSSION V

J. Ransohoff: We will now receive questions on data accumulation and analysis. Dr. Lane?

D. Lane: Do we need more information before we can start stratifying admissions to study, and do you think stratification is appropriate in brain tumor studies?

E. Gehan: We could use this information to stratify patients. I hope that we can get the data on Karnovsky ratings before finalizing them. Since the data demonstrate differences in survival, they have prognostic value. This factor can be continuously refined and data on the Karnovsky rating would add something.

Ransohoff: Is not stratification simply a way to correct for inadequacies of randomization?

Gehan: The most favorable group was the one treated with X-ray. In the unadjusted medians, the patients who received BCNU plus X-ray had a little better survival rate than those who had irradiation alone, but this may be because the latter had a more favorable prognosis than those who received BCNU plus X-ray. This separation allows one to identify BCNU as being effective in these patients.

O. Salazar: We have done something similar in 143 patients with glioblastomas and came up with about the same prognostic factors. Were these patients stratified according to pathology? This poses some difficulty because a striking difference exists between our grade III and grade IV; the 5-year survival was 30% for grade III and zero for grade IV.

Gehan: These patients all had glioblastoma.

M. Mahaley: I am not sure which of Dr. Gehan's figures were restricted to glioblastoma or include the anaplastic gliomas. Both are valid cases for study, but when people talk about grade III and IV glioblastomas, they are not necessarily talking about the same types. The patients with glioblastoma seem to have shorter median survival times than the anaplastic gliomas of all categories grouped together. The tissue pathology does make a difference in survival of probably 10–15 weeks in patients who receive the same therapy, not taking into account other variables, such as age, etc., which we thought were randomly distributed.

C. Leventhal: One purpose of creating this sort of stratification is to develop an adequate historical control group for use in future comparisons. Are your data sufficiently accurate to make this procedure reliable in relation to the parameters you impose on these equations? I have the impression that you are straining for accuracy when you report each factor in 4 or 5 significant figures. What is the real reliability?

Gehan: The number of extra decimal places taken is not pertinent. That was the significance level in this sudy, and if it was significant at less than 0.001, it was highly significant. Those survival curves I presented according to predicted medians may have been misleading. The best fit to the data is obtained if one applies it to the same data from which it was derived. If one does not get a good fit, these factors can be dropped as prognostic indicators. One has to look for consistency among studies as well as significance within studies. In the next study, the patients receiving BCNU plus X-ray should serve as a control group. Our present study demonstrated that a "no treatment" group was not needed, nor perhaps some of the other groups. If, over the course of several studies, the results with the BCNU plus X-ray group remain consistent, these studies would not need to be repeated.

Ransohoff: Dr. Leventhal implied that we might be able to develop predictive factors that could be used from study to study. These patients had glioblastomas or malignant astrocytomas and had been operated on, which separates them from the total group. I do not believe we know whether the predictive factors would be the same for those who did not have surgery.

M. Walker: This study and kind of analysis must be taken in perspective. We collected a tremendous amount of data on these patients. In addition, however, we struggle with certain myths that are passed on from generation to generation, such as "Dominant hemisphere tumors do not do as well as nondominant hemisphere tumors." One of the reasons for this kind of analysis is to verify or dispel these myths. We are still talking about miniscule survival time. Nevertheless, it is important to identify these special subpopulations and to discover factors we may be able to use for stratification. As Dr. Gehan said, we received the first hint of this during the mithramycin study.

Our current investigation supplied a better indicator, and now we must see whether it will hold up when we analyze the next study.

Gehan: For example, age could be used as a stratification variable in future studies based upon the 2 that have been done.

A. Ommaya: Would someone describe the rationale behind this approach? What idea has been tested here?

Gehan: This is an inductive approach, but it is better than the anecdotal pattern which says, "This is good or this is bad," based upon inadequate numbers of patients. Here we can demonstrate marked differences in survival time. I do not regard these as the final choice of factors; they are preliminary choices that can be examined more carefully in future studies.

R. Blasberg: In Dr. Gehan's findings, an unusual factor has appeared, i.e., cranial nerve involvement by glioblastoma multiforme. Have you looked at this more closely? Were the cranial nerves involved in patients with astrocytomas of the brain stem or did this occur in those with cerebral lesions?

Ransohoff: Evidence of increased intracranial pressure (i.e., VI nerve palsy) brought most of these patients to early attention in the same way that seizures do.

Lane: Neurosurgeons, neurologists, chemotherapists, and radiation therapists deal with brain tumors. What may represent a response to one may not be a response to another. How can we reach a position at which the criteria of response are the same for all, whether neurosurgeons or oncologists?

C. Wilson: A cooperative group is studying this problem. It is essential to avoid mistaking one lesion for another and to be able to do a competent neurologic examination. We are not concerned about the minutiae of the neurologic examination but the obviously important things. This cooperative group has written a handbook on neurologic examination for the physician who is following a patient with a brain tumor so that he can record the neurologic findings in a standard form.

The neurologic examination and brain scan should put the right sort of patient into a phase II study. There will be some uncertain cases, but most will be either clear failures or unequivocal responses. I did not discuss the evaluation of the patient with leptomeningiocarcinomatosis, which is an exceedingly difficult disease to follow unless the patient also has a mass.

Ransohoff: Should we not impose a time course in improvement? If the patient shows distinct neurologic improvement after chemotherapy or radiation for 2 weeks, does that constitute a response?

Wilson: That is an excellent point. A phase II study asks, "Does this drug have any activity and in what percentage of patients does it evoke a response?" It does not ask how long or how much activity. The best way of judging the degree of activity is by the length of response. Perhaps the best index is log kill and regrowth, i.e., the length of time you can keep the tumor cell population down. I agree that phase II studies should be reported in duration of response. Is 2 weeks long enough?

Ransohoff: Your question is not related just to phase II studies; it is a general question of great interest to an audience made up of many different kinds of people.

Wilson: In the phase III study, which is no contest and is a job for the statistician, one treats the patient properly and if death is the end point, one waits. If one is using recurrence, the patient has to be followed carefully, and then the statistician determines whether the treatment is good. One does not make a judgment on any individual patient. A phase III study is a committed one, and no judgments are passed until the study is completed. In a phase II study, one looks at the patient week after week after week. How long does a patient have to improve to say that he has responded? With BCNU, we had about a 40% response rate for a median duration of 9 months; with CCNU, it was 40-odd for 6 months; with procarbazine, it was also 40-odd for 6 months. We cannot make distinctions in just a few weeks, because there is such a great variability. I do not recall that we ever asked ourselves that exact question: "What is the minimum time that someone can improve to be called a responder?" Perhaps we have not made a specific decision on this because it has never been a problem.

A. Selker: Let us turn the question around. At what point, in a phase II study after starting a new drug, is there evidence of improvement? Are you talking about one course, two courses, a week, a month?

Wilson: It depends on the length of the course. A course of nitrosourea is 5–8 weeks. Striking changes are often seen in 4–6 weeks. Before a response is seen, we believe the patient must have one full course of therapy and live for 2 months. You can see a response sometimes among all responding patients within 4–6 weeks. If a response is going to occur, it probably will happen in 3 weeks. When Phil Gutin analyzed all our responding patients, 18% had a delayed response after two full courses. They had one course, came back in 6 weeks and had another course; some of these patients went on to become good responders for long periods. One who went well beyond 3 years did not show a response until he had completed three courses. As a rule, we give two full courses before we conclude that the patient is not going to respond to that drug, assuming that he lives through two full courses.

C. Chang: I want to comment on the detection of recurrence. We may believe the tumor is present, but its biologic behavior may be changed, so how are we to decide whether that is a recurrence or not? One must wait for a sequential scan and neurologic changes that accompany the recurrence, and then embark on a new course of treatment. I believe patients with malignant glioma grades III and IV are always going to die, even after a 10- or 15-year period. However, the grade I's are better: in a follow-up of over 100 grade I gliomas, particularly in the optic nerve and chiasmal areas, we have had about 30% survival for 25 years. Those tumors we believe may be cured. Otherwise, patients with grades III and IV all died. Some scientists have reported a 3–5%, 5-year survival, but most of these patients probably die soon after they are reported.

Wilson: Dr. Ransohoff, let me come back to your question. If someone improves for 2 weeks, is he a responder? We do not see our patients between evaluations, which now are usually at either 6 or 8 weeks. We are interested in what happens to them in the meantime, but the computerized data only describe their condition on the return visit.

Ransohoff: That is a 6-week interval at least.

Wilson: Yes.

I. Kricheff: We may be able to learn something about response from a correlation with the radionuclide (RN) scan and perhaps the lack of correlation with the CAT scan. All responders are not alike. Although some patients improve and respond to drugs, they improve clinically and the tumor gets smaller; other individuals show a mass effect that is unchanged, but an enhancement effect that is dramatically changed. It seems to me that the RN scan reflects chiefly the blood–brain barrier; the CAT is looking at the blood–brain barrier, but it is also looking at the mass effect. Could it be that some patients respond to the drug by improvements in blood–brain barrier without any shrinkage of tumor, some respond by tumor shrinkage, and some respond by both?

Wilson: That is a good point. It has been said that the enhancement aspects of the CAT and the RN scans look at the same thing; that is, a permeable blood–brain barrier; yet if it was only blood–brain barrier, one would expect little difference to show.

Ransohoff: Should we call that the tumor–blood barrier?

Wilson: I have confidence in the RN scan because I am familiar with it. It has proved reliable in many hundreds of patients. The CAT scan is more complicated; there are many more varieties and we do not yet feel comfortable using it as an indication of response. As the CAT scanning improves, we will gain more confidence. I do not believe we will discard RN scans because they provide another way to look at the tumor. Until the brain tumor problem is solved, we will continue to use both.

Brain Tumor Study Group: A Survey of Current Activities[1]

Michael D. Walker[2]

ABSTRACT—Improvement in the treatment of patients harboring malignant glioma will probably be seen as small incremental changes as new modalities of treatment are proposed, tested, and substantiated. Uncontrolled phase II studies may provide hints of efficacy. However, the results must be substantiated in carefully controlled phase III evaluations. The Brain Tumor Study Group of the National Cancer Institute has demonstrated that mithramycin is not effective in the treatment of malignant glioma and the overall median survivorship experience is no different than the 23 weeks found in historic controls. Radiotherapy can bring about a meaningful increase in survival as can 1,3-bis(2-chloroethyl)-1-nitrosourea. The combination appears to produce more long-term survivors than either treatment above. Methyl-1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea does not appear to be additive to radiotherapy but is more effective than no therapy at all. Studies of the oncolytic effect of corticosteroids in contradistinction to their cerebral edema controlling effects are being done and will provide meaningful data on this important symptomatolytic therapy.—Natl Cancer Inst Monogr 46: 209–212, 1977.

The literature contains many reports of various modes of treatment of malignant brain tumor (*1*), most of which are uncontrolled phase II evaluations of a specific mode of therapy performed without the use of either historical or concurrent controls. These studies usually are done on patients who have recurrent disease and, therefore, have a large tumor burden, who are sick and debilitated from the effects of the tumor, and have a limited life expectancy (*2*). The data presented in the literature vary widely in the parameters analyzed, and the studies are difficult to assess and compare.

An evaluation of the literature presents major difficulties related to such factors as the method of patient selection, the biologic characteristics of the tumor as it may be reflected in histopathology, the extent of prior therapy, and a multitude of other variables that we have yet to define clearly. These factors may have a greater impact on the individual's response to therapy or the survival of treated patients than many of the therapies now in use. Therefore, one of the major requirements in evaluating any form of treatment is accurate data concerning patient population characteristics and how they may interact with a given therapeutic modality. If the treatment that we use would prove a "quantum jump" in response rate or survival, i.e., a threefold to tenfold increase or the "Lazarus effect" (a truly remarkable therapeutic effect), controlled clinical trials would be unnecessary. Since these responses have not been encountered during the last 20 years of therapeutic effort in any form of cancer, let alone brain tumor, we cannot expect to dispense with controlled trials in the immediate future.

A pressing need exists for highly controlled studies that clearly define the characteristics of the patient population and that will develop therapeutic data which, though at first may not reflect dramatic gains, will provide a basis for the planning of future studies. The best example of this approach is the slow but sure progress that has been achieved in the treatment of acute leukemia over the last two decades (*3*). Hence the chief hope of progress apparently is in an incremental improvement in therapeutic approaches.

The Brain Tumor Study Group (BTSG) is a cooperative endeavor undertaken by the National Cancer Institute and participating institutions in leading neuro-oncologic centers (table 1). It is the express intent that each protocol undertaken by the Group contain two elements: It should address itself to a basic question germane to the study of the disease entity with which we deal; in addition, the protocols should evaluate specific modes of therapy and provide detailed quantitative data relative to therapeutic effectiveness and toxicity. Gehan's (*4*) data on prognostic factors represent a first attempt to define specifically those patient or tumor characteristics that may directly influence survival, positively or negatively. The development of these data and the application of these factors will provide better techniques for stratifying and evaluating various therapies. The data that Mahaley and Vogel (*5*) provided concerning the types of histopathology and the possible toxic effects seen in normal tissue secondary to therapy are other examples of significant and important information generated from these studies. However, the major thrust is to develop acceptable programs of therapy by which we can bring about qualitative as well as quantitative improvement in the survival of patients with malignant brain tumor.

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The first study performed by the BTSG evaluated the efficacy of mithramycin as a specific mode of therapy (*6*). Since several phase II studies indicated that this drug has value in the treatment of malignant glioma, it is important to define its therapeutic efficacy (*7*, *8*). The specific aims, basically, were to demonstrate that controlled, prospective, randomized studies could be done in the treatment of malignant glioma and that meaningful data could be generated therefrom. Within 3 weeks of definitive surgery and the establishment of the diagnosis of anaplastic gliomas, patients were randomized to receive mithramycin (25 μg/kg/day iv over 8 hr every 21 days), or no mithramycin and continued best conventional care. Therefore, all patients would receive the usual therapy administered in the treatment of this disease, but half the randomized population would receive mithramycin in addition. Because of the highly variable and unpredictable toxicity of mithramycin, all patients were carefully monitored, and those who did not receive this drug were to be evaluated and followed in the same fashion. The most significant information derived from this study was that malignant glioma can be evaluated in prospective, controlled, randomized studies similar to those used in the evaluation of other forms of neoplastic disease. This finding is far more important than the demonstration that mithramycin is of no value in the treatment of malignant glioma.

Approximately 116 patients were entered in the randomized population, of which 96 were considered within the Valid Study Group. Most of the protocol violations were failure to adhere to the strict pathologic criteria or to other variations not allowed in the protocol. The median survival of those treated with mithramycin was 21 weeks from time of randomization, whereas those who did not receive it had a median survival of 26 weeks. No significant difference was noted between the two groups, and the median survival for the 96 patients was 23 weeks. A careful compilation and analysis of the historical, symptomatic, and neurologic data available on these patients and the histopathology of their tumors provided a clear definition of the patient population under study. Factors such as duration of symptoms, concurrent radiotherapy, and various signs and symptoms were specifically related to survival and hence may be considered as important variables. Mithramycin in the doses used gave rise to a variety of serious hematologic and hepatic dysfunctions (*6*); for this reason, mithramycin should no longer be considered for the treatment of malignant glioma.

TABLE 1.—*Institutions participating in the BTSG studies*[a]

Bowman Gray School of Medicine
Indiana University School of Medicine
Ohio State University Hospital
University of California Medical Center
Duke University Medical Center
New York University Medical Center
Sloan-Kettering Memorial Hospital
University of Kentucky Medical Center
St. Louis University School of Medicine
University of Tennessee Center for the Health Sciences
Montefiore Hospital of the University of Pittsburgh
University of California, Los Angeles
Universitá Di Pavia[b]
Baltimore Cancer Research Center
University of Connecticut
Mayo Clinic
Roswell Park Memorial Institute

[a] All but the last three are current participating institutions in the BTSG.

[b] This is located in Pavia, Italy.

During the course of these studies, the nitrosoureas, the first of the rationally developed and designed chemotherapeutic analogs, were being evaluated (*9–11*). These drugs possess characteristics believed desirable in the treatment of malignant brain tumor: a high degree of lipid solubility, a low degree of ionization, and a small molecular weight (*12*). From the literature and the mithramycin study, it was apparent that radiotherapy played an important role in the treatment of malignant glioma. Qualitative information on its efficacy and therapeutic responses was available, but precise quantitative data were not (*13*, *14*).

The next study set out to define the specific increase in median survival in patients with malignant glioma that could be expected from the use of radiotherapy. 1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) was chosen as the chemotherapeutic agent because uncontrolled studies indicated its efficacy, and its value had been established in other forms of cancer (*10*, *11*). BCNU (80 mg/m^2/day on 3 successive days every 6–8 weeks), radiotherapy (5,000–6,000 rads through bilateral opposing ports over 5–7 weeks), and the combination of BCNU and radiotherapy (in the above doses) were compared with the usual conventional care without antineoplastic chemotherapy or radiotherapy. The significance of corticosteroids in the treatment of malignant glioma was becoming apparent, and many investigators believed that no other drug had a more marked effect in the clinical management of patients with this disease. For this reason, this protocol carefully controlled the use of corticosteroids so as to avoid inadvertently biasing the data by its use.

Of the 303 patients entered in the randomized population, 225 are within the Valid Study Group. Patients were disqualified usually because 1) their tumors did not fit the protocol requirements of histopathology, 2) use of corticosteroids was excessive, or 3) incorrect therapy was given. However, any patient who received any amount of BCNU or radiotherapy was included in the Valid Study Group. It became apparent that patients who succumbed to their disease within a few weeks did not have a reasonable trial of therapy and, therefore, the Adequately Treated Group (containing 167 patients) was established. This included all patients who received 5,000 or more rads of radiotherapy and two or more courses of BCNU, and survived a minimum of 8 weeks, i.e., the time required to deliver this therapy if they had been randomized to those therapeutic arms. Since most of the patients in this study have succumbed to their disease, only small changes in the data base are anticipated. Within the Valid Study Group, the median survival of those who did not receive chemotherapy or radiotherapy was 14 weeks. Those who received at least 1 dose of BCNU had a median survival of 18.5 weeks, and those who received at least 1 dose of radiotherapy had a median survival of 36 weeks, whereas those who had any treatment in the combination arm had a median survival of 34.5 weeks.

The Adequately Treated Group, a subset of the Valid Study Group, provides more specific information concerning the efficacy of these modes of therapy. Within the Adequately Treated Group, those who received the best conventional care (including a minimum guaranteed survival time of 8 weeks) had a median survival of 17 weeks, whereas patients to whom two or more courses of BCNU were administered and those who received 5,000 or more rads of radiotherapy had median survivals of 25 and 37.5 weeks, respectively. The combination of BCNU and radiotherapy provided a median survival of 40.5 weeks. This study demonstrated that all forms of therapy provided some improvement in the median survival of patients with malignant glioma. However, BCNU alone was less effective than radiotherapy alone. Combination therapy when compared with radiotherapy only provided a small increase at the median survival point; at the end of 18 months, virtually all the patients who received a single mode of therapy had succumbed to their disease, whereas approximately one-fifth of those receiving both BCNU and radiotherapy survived.

The comparability of treatment must be examined to determine if a significant, as well as an equivalent, amount of therapy was used. Sixty-six percent of the patients who were given BCNU and 81% of those administered the combination received two or more courses of chemotherapy. An average of 5,200 rads was given to all patients receiving radiotherapy, and 76% of those treated with radiotherapy alone received 5,000 rads or more as compared with 84% of those given the combination. This study clearly indicated the quantitative value of radiotherapy and BCNU chemotherapy.

The following study compared the difference in efficacy between the newest of the nitrosoureas, 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU), 220 mg/m^2 given orally every 6–8 weeks, 6,000 rads whole-brain irradiation through bilateral opposing ports over 6–8 weeks, Me-CCNU and radiotherapy (in doses noted), with the best treatment arm of the previous study, i.e., BCNU and radiotherapy. Although this study is closed to the accrual of new patients, therapy is still going on and information is being added to the data base. Therefore, these results must be considered as a preliminary interim analysis. Three hundred forty-nine patients have been entered in the available randomized population. In February 1976, 263 patients were entered in the Valid Study Group and approximately two-thirds of the patients have succumbed to their disease. A projected analysis (Kaplan and Meir technique) indicates that the median survival of those who have been treated with Me-CCNU alone or Me-CCNU and radiotherapy is approximately 31 weeks. Those who were given radiotherapy alone have a median survival of 36 weeks, whereas those who received BCNU and radiotherapy have a median survival of 51 weeks (*15*). An analysis of factors that might influence response to treatment did not reveal any significant difference between the various patient populations under study. The final analysis of this study will await the results of further treatment of those patients who are still alive.

It is interesting to speculate on the significance of the differences in median survival between patients treated with BCNU and radiotherapy in this continuing study and the previous one (34.5

weeks vs. 51 weeks). The differences are probably secondary to the enthusiasm that a positive study generates for a given mode of therapy. Although the mithramycin study failed to demonstrate that the drug had any value, the BCNU-radiotherapy study was clearly positive. This provided workers with the incentive to treat patients more diligently and more effectively in the succeeding study, and the results of this are apparent, in part, in an improved survival.

In summary, the BTSG has performed carefully controlled, prospective, randomized studies that will provide basic information about malignant glioma and therapeutic approaches to this tumor. A considerable amount of peripheral information is being collected which is of great value in defining more clearly the patient populations that should be treated. In addition, the methods of study, techniques of operating the central office, accruing patients, and processing data have been more clearly understood. The specific value of BCNU, radiotherapy, and Me-CCNU in the treatment of malignant glioma has been defined.

The study currently being undertaken by the BTSG will address itself to the question of the antineoplastic (as opposed to the anti-edema) effect of corticosteroids. This research has the advantage of being "positive" in its results regardless of the outcome, for if it is demonstrated that corticosteroids do not have an oncolytic effect and, therefore, are presumed to exert their effects through the control of cerebral edema, we will be able to use them to increase the comfort and well-being of our patients without fear that such use will bias our studies. On the other hand, if it is demonstrated that corticosteroids have a definitive oncolytic effect, we will have another relatively nontoxic, marrow-sparing drug with which to treat this disease. In addition to evaluating the use of corticosteroids, the efficacy of procarbazine will be determined. Since these studies were begun in 1976, any conclusions about them are approximately 2 years away.

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Therapeutic Trials in the Management of Metastatic Brain Tumors by Different Time/Dose Fraction Schemes of Radiation Therapy[1, 2]

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ABSTRACT—Two consecutive studies have been performed by the Radiation Therapy Oncology Group on patients with metastatic brain tumors. Approximately 1,000 patients were entered into each trial. Treatment schedules of varying dose–time fractionations were used. Results were evaluated on the basis of improvement in general performance and neurologic function status. Survival also was recorded. Patients who received corticosteroids in conjunction with radiation therapy experienced a more rapid improvement in neurologic function than patients who did not receive steroid therapy. This was noted particularly in patients with poor neurologic function status. However, by the 4th week this difference had disappeared. Survival times were not altered by the addition of steroid therapy. Preliminary results of the second study suggest that ultra-short fractionation schedules are less effective than the longer ones used in the first study. A dose of 3,000 rads given in 10 equal fractions for a period of 2 weeks appeared to be the most satisfactory schedule for most patients with intracranial metastases.—Natl Cancer Inst Monogr 46: 213–221, 1977.

Approximately half of all intracranial neoplasms seen in a general hospital are metastatic tumors. They occur commonly in patients with lung or breast cancer, and once symptomatic, they lead to rapid incapacitation of the patient and to death. The value of radiation therapy in the treatment of metastatic brain tumors was reported by Chow et al. (*1*) in 1954 and later by Chu and Hilaris (*2*) in 1961. A number of reports since then have indicated the value of radiation therapy in the management of such metastatic tumors and have stressed the fact that patients treated by irradiation can, in many instances, be restored to good function and have prolonged survival (*3–5*).

Surgical management enters into the treatment of these patients in two ways. In a number of patients, symptoms of intracranial mass are present without any indication of a primary tumor elsewhere, and the diagnosis is made by craniotomy. Secondly, surgical management is of value in tumors in certain sites that have a propensity for solitary metastasis and a long natural history, such as primary tumors of the colon and corpus uteri. If any doubt exists as to whether the intracranial lesion is related to a malignant primary tumor when there is no other evidence of metastasis and some time has elapsed since treatment of the primary, surgical exploration is required because other intracranial mass lesions, including benign tumors, may occur in such patients.

Because of the role that radiation does play in the treatment of metastatic brain tumors, the Radiation Therapy Oncology Group (RTOG) has undertaken two consecutive clinical trials to evaluate different time and dose fraction regimens in their treatment. A description of these trials and their results (preliminary in the case of the second study) is the subject of this report.

FIRST STUDY ON TREATMENT OF METASTATIC BRAIN TUMORS BY RADIATION THERAPY

The first trial on metastatic brain tumors by radiation therapy was initiated by the RTOG in January 1971; accession of cases was completed by August 1973. A total of 1,021 patients was entered into this trial; 40 institutions (listed in the "Appendix" with the names of their principal investigators) participated. This trial attempted to compare different doses of total brain irradiation delivered in different fractions and during three different intervals (text-fig. 1). In addition, an optional treatment arm used a single high dose to the whole brain. The objective of this study was to determine if short treatment schedules were as efficacious as the longer ones, with consequent savings in time and costs, both to patients and radiation therapy departments. Evaluation took place on the basis of improvement in neurologic status and general condition. Survival time was

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[1] Presented at the Symposium on Modern Concepts in Brain Tumor Therapy: Laboratory and Clinical Investigations, held in Atlanta, Ga., February 26–28, 1976, and sponsored by the Clinical Investigation Branch and the Cancer Clinical Investigations Review Committee, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. 20014.

[2] Supported by Public Health Service grant CA 12248 from the National Cancer Institute.

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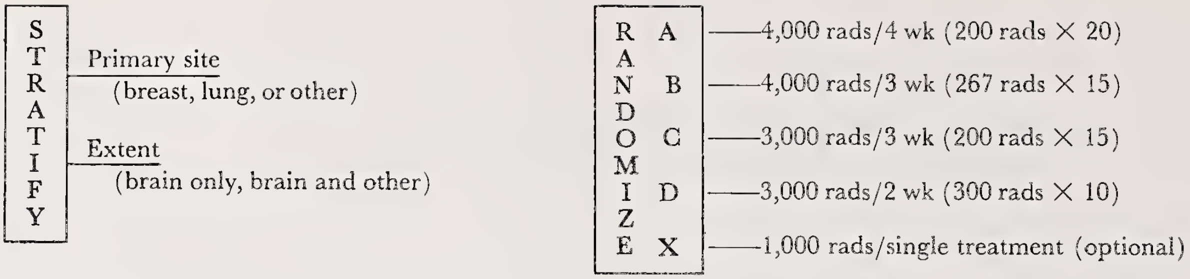

STRATIFY
Primary site
(breast, lung, or other)
Extent
(brain only, brain and other)
RANDOMIZE
A 4,000 rads/4 wk (200 rads × 20)
B 4,000 rads/3 wk (267 rads × 15)
C 3,000 rads/3 wk (200 rads × 15)
D 3,000 rads/2 wk (300 rads × 10)
X 1,000 rads/single treatment (optional)

TEXT-FIGURE 1.—Schema for the first study of the palliation of brain metastasis in 1,021 patients conducted by the RTOG, initiated in January 1971 and closed in August 1973.

TABLE 1.—*Primary site, extent of metastases, and treatment with percentage distribution within treatment*[a]

Primary site, and extent of metastases[b]	Treatment[c]					Total
	A	B	C	D	X	
Breast						
Alone	3.4	4.1	3.8	4.4	—	3.8
With	16.4	13.6	14.8	11.9	8.0	14.1
Subtotal	19.8	17.7	18.6	16.3	8.0	17.9
Lung						
Alone	46.2	42.3	44.5	46.3	44.0	44.8
With	14.7	20.0	16.5	17.1	28.0	17.3
Subtotal	60.9	62.3	61.0	63.4	72.0	62.1
Other						
Alone	5.9	5.9	9.7	7.9	8.0	7.4
With	13.4	14.1	10.6	12.3	12.0	12.6
Subtotal	19.3	20.0	20.3	20.2	20.0	20.0
All sites						
Alone	55.5	52.3	58.1	58.6	52.0	56.0
With	44.5	47.7	41.9	41.4	48.0	44.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
No. of patients	238	220	236	227	25	946

[a] Reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

[b] Definition of extent of metastases: Brain alone: patient had brain metastases and no other metastatic site; brain with: patient had metastases to the brain and at least one other site.

[c] *See* text-figure 1 for definitions.

measured, although this end point, of course, depended on other uncontrolled factors, such as extent of primary or metastatic disease elsewhere in the body. The addition of steroid therapy also was evaluated.

The diagnoses of the patients were established on the basis of their clinical symptoms, radiologic investigation by arteriograms or pneumoencephalograms, and radioisotope brain scans. Histologic confirmation was obtained only in those patients who underwent a surgical procedure. For the randomization of patients into treatment arms, cases were stratified by site of primary tumor (breast, lung, and other) and by presence or absence of metastases outside the brain. Patients were also stratified by whether or not corticosteroids were used; the use of steroids was not and could not be controlled. The distribution of cases by primary site and extent of metastases or treatment location is shown in table 1. In nearly two-thirds of the patients, the lung was the primary site and over 70% of the patients with primary lung cancer had no other metastasis, whereas only 20% of the patients with primary breast cancer were without extracranial metastasis. Table 2 describes the status of the tumor at the primary site; most of the patients with breast cancer had no evidence of primary tumor, whereas two-thirds of the patients with lung cancer did. Table 3 indicates the number of patients who underwent surgical procedures; these constitute only 12% of the total in the study and include those having either biopsy or gross removal of the tumor.

Attempts were made to score the patient's improvement on a subjective and an objective basis. Table 4 gives the percentage of patients

TABLE 2.—*Primary site, status of primary, and treatment with percentage distributions within treatment*

Primary site, and status of primary[a]	Treatment[b]					Total
	A	B	C	D	X	
Breast						
Absent	6.8	15.9	15.7	14.5	8.0	15.5
Present	2.1	0.5	2.1	1.8	—	1.6
Unknown	0.8	1.4	0.8	—	—	0.7
Lung						
Absent	19.3	17.7	17.8	21.6	24.0	19.2
Present	39.9	42.7	41.1	38.3	48.0	40.7
Unknown	1.7	1.8	2.1	3.5	—	2.2
Other						
Absent	12.6	11.8	10.6	11.0	8.0	11.4
Present	6.7	6.4	8.5	6.6	12.0	7.2
Unknown	—	1.9	1.3	2.7	—	1.4
All sites						
Absent	48.7	45.5	44.1	47.1	40.0	46.2
Present	48.7	49.5	51.7	46.7	60.0	49.5
Unknown	2.6	5.0	4.2	6.2	—	4.3
Total	100.0	100.0	100.0	100.0	100.0	100.0
No. of patients	238	220	236	227	25	946

[a] Status of primary indicates initial assessment.

[b] *See* text-figure 1 for definitions.

TABLE 3.—*Surgery on the brain*[a, b]

Type of surgery	Treatment[c]					Total[c]
	A	B	C	D	X	
	No.	No.	No.	No.	No.	No.
None	204 (86)	184 (83)	197 (84)	200 (88)	21 (84)	806 (85)
Biopsy	12 (5)	8 (4)	13 (5)	6 (3)	1 (4)	40 (4)
Gross removal	16 (7)	22 (10)	17 (7)	18 (8)	3 (12)	76 (8)
Not reported	6 (2)	6 (3)	9 (4)	3 (1)	0 –	24 (3)
Total	238 (100)	220 (100)	236 (100)	227 (100)	25 (100)	946 (100)

[a] *Numbers in parentheses* indicate percentages (rounded).
[b] Data are reprinted with permission of the publisher of *Seminars in Oncology* (*6*).
[c] *See* text-figure 1 for definitions.

TABLE 4.—*Symptomatic change*[a]

Symptom	Change, %				No. of patients evaluated
	Complete	Partial	None	Worse	
Convulsions	67	24	5	4	188
Headache	54	31	7	8	487
Motor loss	34	42	13	11	445
Impaired mentation	32	40	10	18	347

[a] Data are reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

TABLE 5.—*General medical status before, during, and after treatment*

Status	Class	Description
General performance	1	Normal
	2	Symptoms, but ambulatory
	↓	↓
Neurologic functional	1	Able to work; etc.
	2	Able to be at home, etc.
	↓	↓

showing a degree of improvement or lack of it for major symptom groups. Between 70 and 90% of the patients had either partial or complete improvement in symptoms. For this assessment, we used two parameters to summarize the patient's general medical status (general performance and neurologic function status) before, during, and after treatment. The classification scales for these two states are given in table 5. Table 6 shows the relationship of primary site to change in neurologic class.

The use of corticosteroids was not controlled in this study but it was a factor we considered in the data analysis. Table 7 gives the percentage of patients within each treatment arm of the study to whom corticosteroids were administered. One-quarter of all patients received no steroids, and in almost 40%, steroid therapy was initiated less than 1 week before protocol therapy. In general, the patients with more severe symptoms were given corticosteroids. Tables 8 and 9 list the percentages of patients with overall change in neurologic function related to steroid administration. In patients with an initial neurologic function status of 2 or 3, neurologic function improved more quickly in those given steroids; by the 4th week, no difference was noted in improvement from those not given the drugs. Again at the 8th week, no difference was observed between these 2 groups. Table 10 indicates that, overall, neurologic function status improves more often with steroid administration than without, but only for a short time and in patients with poorer neurologic function status. Although these findings are by no means conclusive, they indicate that corticosteroids may be useful adjuncts to radiation therapy, particularly in the treatment of patients with severe symptoms of intracranial metastasis. However, we have no indication that the steroids have any antineoplastic activity of their own because by the 4th week after the initiation of therapy, no advantage can be shown in those patients on steroid therapy, nor is their median survival longer.

Table 11 establishes risk groups according to neurologic function and general performance. Survival curves are plotted in text-figures 2 and 3 according to the treatment arm used and the risk group, respectively. Few patients were available for the single-dose treatment (an optional arm of the study), and thus no conclusions can be drawn concerning this treatment (text-fig. 2). The results for the other 4 groups are similar, with perhaps minimal difference in improvement between the group treated by 4,000 rads in 3 weeks and that

TABLE 6.—*Influence in percent of primary site on change in neurologic class*[a, b]

Change	Breast	Lung	Other	Total
Improved	41.5	41.7	44.4	42.2
None change[c]	31.3	29.2	29.0	29.5
Progression	27.2	29.2	26.6	28.3
Total	100.0	100.0	100.0	100.0

[a] Data are reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

[b] Percentages have been rounded.

[c] Many in "no change" had symptom improvement.

TABLE 7.—*Corticosteroids and treatment with percentage distribution within treatment*[a]

Steroid status	Treatment[b]					Total
	A	B	C	D	X	
No steroids	25.0	23.1	28.0	24.8	12.0	24.9
Initial administration:						
> 1 week before protocol therapy	23.2	21.6	21.1	22.9	28.0	22.4
< 1 week before protocol therapy	37.1	41.8	40.8	39.3	44.0	39.8
After protocol therapy	4.0	4.3	5.5	2.8	4.0	4.2
Other steroids	10.7	9.1	4.6	10.3	12.0	8.8
Total	100.0	100.0	100.0	100.0	100.0	100.0
No. of patients	224	208	218	214	25	889

[a] Percentages have been rounded.

[b] *See* text-figure 1 for definitions.

given 3,000 rads in 2 weeks over the other 2 groups. With regard to the risk groups (text-fig. 3), it is clear that median survival is markedly longer in those patients in the low-risk than in the high-risk groups, but this difference ceases at about 50 weeks. Finally, table 12 correlates the cause of death to the length of survival. Whereas death in over 60% of those patients dying within 5 weeks is related to brain metastasis, this is the cause in less than 50% of those with longer survival times. Most of the other deaths are due to uncontrolled cancer at sites other than the brain. Our conclusions are summarized in table 13.

In summary, this study indicates that 3,000 rads given to the whole brain in 10 treatments for a period of 2 weeks is a satisfactory way of treating brain metastasis. This reduction in overall time of treatment from 4 to 2 weeks is not only an advantage to the patient, in that it shortens his in-hospital stay or overall treatment time, but is also of major socioeconomic value. It is estimated that the reduction in cost at a national level can amount to a savings of between 5 and 10 million dollars a year.

TABLE 8.—*Percentage of patients with change in neurologic function related to steroid administration*[a, b]

Change	Week of evaluation				Total
	1	2	3	4	
Initial neurologic function					
Improvement	—	37.9	61.3	57.1	42.1
No change	36.2	33.3	20.6	42.9	29.6
Progression	63.8	28.7	18.1	—	28.3
Total	100.0	100.0	100.0	100.0	100.0
No steroids while on study					
Improvement	—	34.7	52.6	44.4	34.0
No change	33.3	36.7	31.6	55.6	35.5
Progression	66.7	31.6	15.8	—	30.5
Total	100.0	100.0	100.0	100.0	100.0
Steroids while on study					
Improvement	—	40.0	64.9	61.5	45.5
No change	32.5	33.0	18.3	38.5	26.6
Progression	67.5	27.0	16.9	—	27.9
Total	100.0	100.0	100.0	100.0	100.0

[a] Data are reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

[b] Percentages have been rounded.

TABLE 9.—*Percentage of patients with improved neurologic function by steroid status and initial neurologic function*[a]

Initial neurologic function and steroid status	Week of evaluation				Week
	1	2	3	4	8
Status 2					
No steroids	3.0	6.7	16.5	30.9	34.4
Steroids	8.3	14.0	23.7	26.2	36.9
Status 3					
No steroids	19.4	25.0	46.7	76.2	75.0
Steroids	31.0	42.7	58.3	63.1	72.7

[a] Data are reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

SECOND STUDY ON TREATMENT OF METASTATIC BRAIN TUMORS BY RADIATION THERAPY

Upon reviewing the results of the first study, the RTOG decided to investigate the possibility of using even shorter treatment schedules and comparing them with the best schedules obtained in the first study. The second one was subdivided into groups A and B. In A, the treatment schedules of 4,000 rads in 3 weeks and 3,000 rads in 2 weeks, used in the first study, were compared with a new schedule of 2,000 rads given in 5 fractions over 1 week. In B, this new schedule of 2,000 rads in 1 week was compared with a schedule of 1,200 rads given in 2 fractions of 600 rads each in 2 days. The institutions participating in the RTOG made a choice of which arm of the protocol (A or B) to enter before the accessioning of patients. The same general considerations and conditions obtained in the second study as in the first, the randomization of patients to treatment being based on stratification by site and institution.

TABLE 10.—*Median survival times in wk by steroid status and the initial neurologic function class*[a]

Steroid status	Initial neurologic function				Median value for steroid groups
	1	2	3	4	
No steroids	35.5	17.6	14.4	2.2	17.3
Steroids	28.6	15.0	12.6	5.7	14.6
Median value for class	29.6	15.9	12.9	4.7	14.9

[a] Reprinted with permission of the publisher of *Seminars in Oncology* (*6*).

TABLE 11.—*Risk groups according to neurologic function and general performance*

Risk group	Neurologic function	General performance	No. of patients	Median survival, wk
1	1	1–2	121	26.9
2	2–3	2	355	19.6
3	2–3	3–5	339	13.5
4	4	5	35	4.6

TABLE 12.—*Correlation of cause of death and survival, %*

Cause of death	Survival time, wk[a, b]					Total (746)
	≤ 5 (158)	6–10 (126)	11–15 (105)	16–20 (74)	≥ 21 (283)	
Not related to disease	4.4	3.2	1.9	4.1	2.8	3.2
Brain metastases	61.4	40.5	46.7	51.4	46.3	49.1
Cancer at other site	20.3	43.7	36.2	36.5	35.3	33.8
Unknown	13.9	12.7	15.2	8.1	15.5	13.9
Total	100.0	100.0	100.0	100.0	100.0	100.0

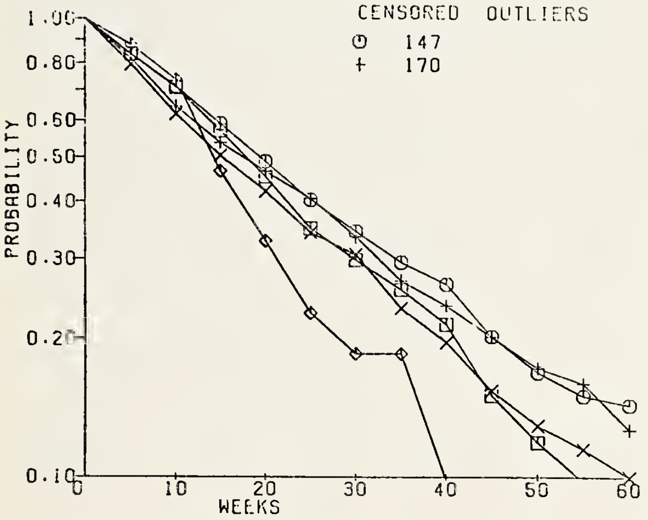
[a] *Numbers in parentheses* refer to number of patients.

[b] Percentages have been rounded.

This study began in September 1973, and accession was completed in January 1975. Randomization was arranged in such a way that in group A twice as many patients were randomized to the new arm (2,000 rads in 1 week) as to each of the other 2 arms (3,000 in 2 weeks and 4,000 in 3 weeks), for which results had already been obtained in the first study. In all, 918 patients were entered in group A and 78 in group B. Text-figure 4 illustrates the schema for this study. Table 14 shows the distribution of patients (which is similar to that of the first study), by primary site and treatment groups.

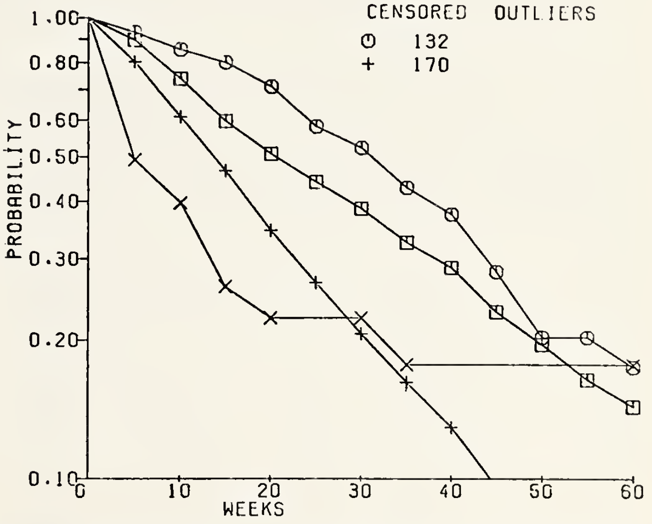
Conclusions discussed here are entirely preliminary since accession to this study has only just been concluded. Table 15 shows the influence of steroids on improvement in neurologic function in patients with an initial status of 2, 3, or 4. Again, there is more rapid improvement of patients given steroids but by the 4th week of assessment, this difference essentially has disappeared.

Survival curves for group A patients are depicted in text-figure 5. In reporting the survival in the second study, the arms have been blinded.



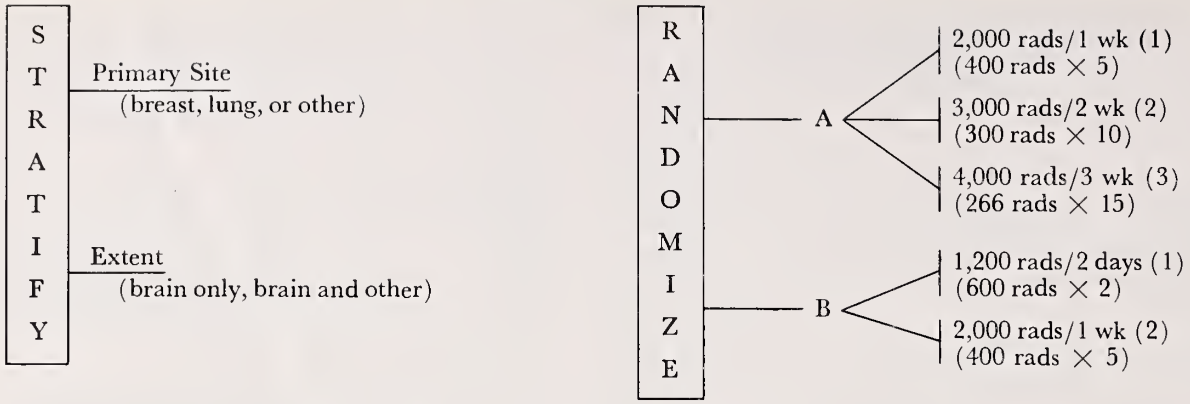
TEXT-FIGURE 2.—Survival of patients treated according to treatment arm of the study. "Outliers": 2 patients with exceptionally long survivals.

	Censored	Deaths	Total
⊙ Treatment A	33	196	229
⊡ " B	30	179	209
+ " C	28	192	220
X " D	29	191	220
◇ " X	3	21	24



TEXT-FIGURE 3.—Survival of patients according to risk group. "Outliers": 2 patients with exceptionally long survivals.

	Censored	Deaths	Total
⊙ Risk group 1	31	90	121
⊡ " " 2	49	306	355
+ " " 3	33	306	339
X " " 4	6	29	35



TEXT-FIGURE 4.—Schema for the second study of the palliation of brain metastasis conducted by the RTOG.

TABLE 13.—*Results and impact of first study*

Results:

1) Treatment arms B (4,000 rads in 3 wk) and D (3,000 rads in 2 wk) led to most favorable results.
2) In 40% of treated patients, ultimate death was not related to brain metastasis.
3) Steroid administration led to more rapid improvement, particularly in patients whose neurologic function status was poor. This advantage was lost by the 4th wk of evaluation.

Impact:

1) Palliative radiation therapy has been demonstrated to be valuable in treatment of brain metastasis.
2) Adoption of shorter treatment regimens (2 wk vs. 4 wk) throughout the United States will lead to savings to the economy of 5–10 million dollars.
3) Research to determine if even shorter courses of radiation therapy are equally effective (second study in progress, September 1973–December 1975).

It now begins to appear that some separation of the curves occurred. This is particularly noticeable when survival curves are plotted for patients with initial neurologic function status 1 (text-fig. 6) and to lesser extent, with neurologic function status 2 (text-fig. 7). The same apparent effect was seen in plotting survivals in patients with metastasis from a primary lung cancer with an initial neurologic function 2 (text-fig. 8). Text-figure 9 shows survival distribution in group B patients; too few patients are available to draw any conclusion. Table 16 lists the causes of death in group A patients.

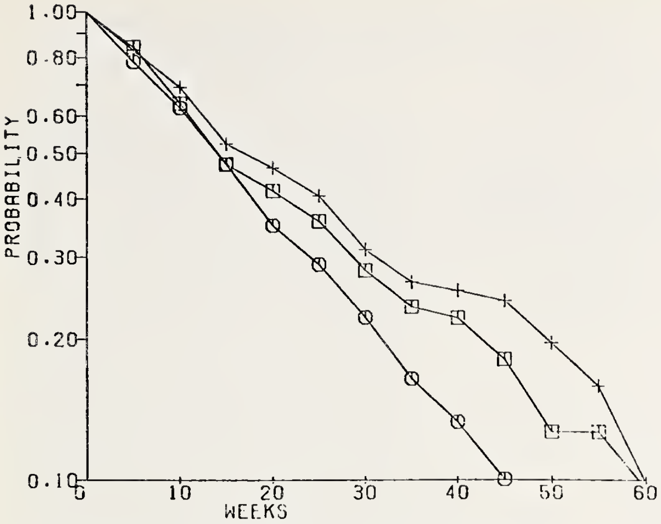
Whereas the results are preliminary, it does appear as though short treatment times, a week

TABLE 14.— *Distribution of patients by site of primary and treatment*[a]

Primary site and extent of metastases[b]	Group A					Group B			
	Treatment			Total	Percent	Treatment		Total	Percent
	T-1	T-2	T-3			T-1	T-4		
Breast									
Alone	17	9	5	31	3.6	1	2	3	4.0
With	51	26	27	104	12.2	2	0	2	2.7
	68	35	32	135	15.8	3	2	5	6.7
Lung									
Alone	166	76	78	320	37.5	17	22	39	52.0
With	79	43	45	167	19.6	7	5	12	16.0
	245	119	123	487	57.1	24	27	51	68.0
Other									
Alone	42	22	24	88	10.3	0	2	2	2.7
With	68	40	36	144	16.9	7	10	17	22.7
	110	62	60	232	27.2	7	12	19	25.4
All sites									
Alone	225	107	107	439	51.4	18	26	44	58.7
With	198	109	108	415	48.6	16	15	31	41.3
Total	423	216	215	854	100.0	34	41	75	100.0

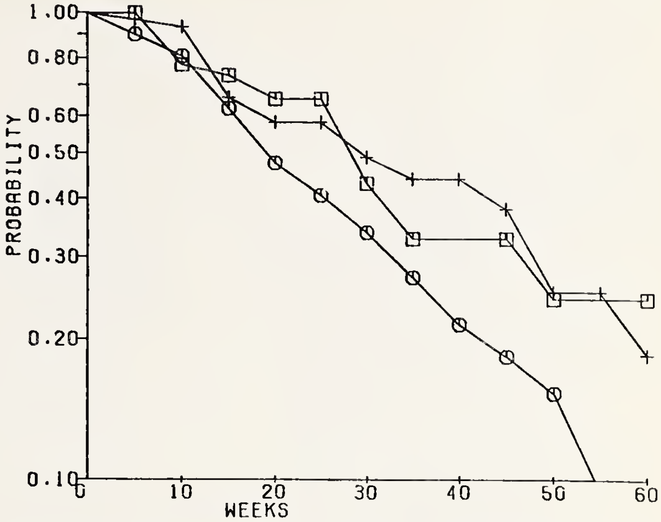
[a] Percentages have been rounded.

[b] Extent of metastases: Brain alone: patient had brain metastases and no other metastatic site. Brain with: patient had metastases to brain and at least one other site.



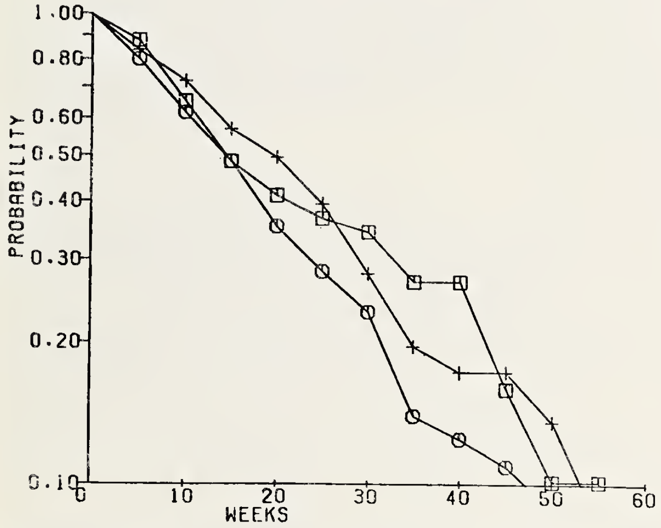
TEXT-FIGURE 5.—Survival of patients treated by treatment arms in group A.

	Censored	Deaths	Total
⊙ Treatment T-1	109	277	386
⊡ " T-2	61	128	189
+ " T-3	75	123	198



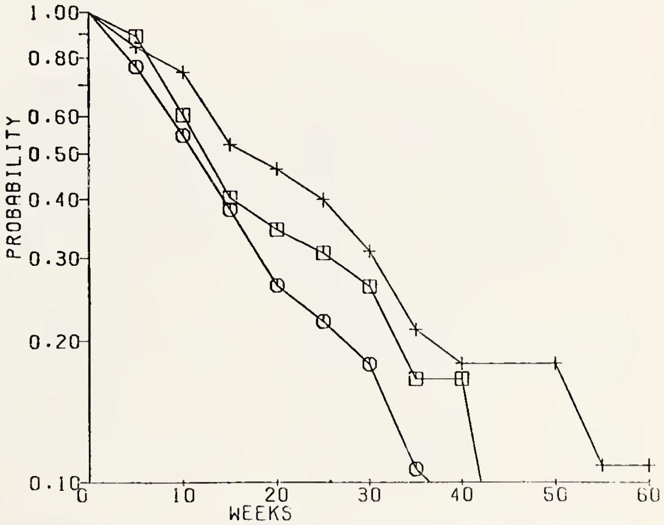
TEXT-FIGURE 6.—Survival of group A patients with initial neurologic function status 1.

	Censored	Deaths	Total
⊙ Treatment T-1	17	44	61
⊡ " T-2	11	19	30
+ " T-3	13	19	32



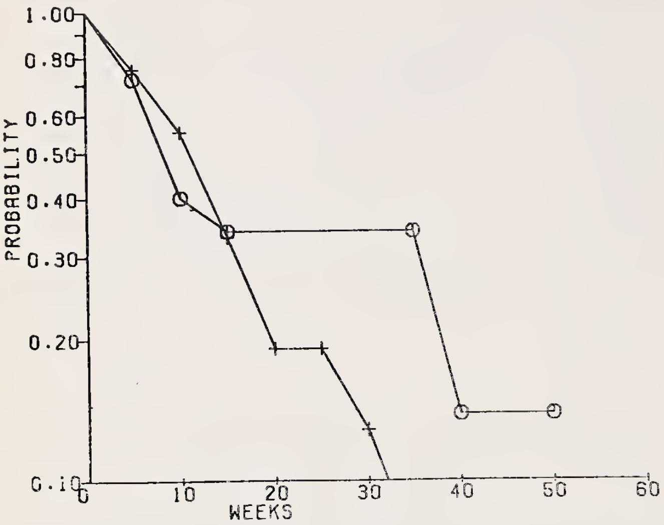
TEXT-FIGURE 7.—Survival of group A patients with initial neurologic function status 2.

	Censored	Deaths	Total
⊙ Treatment T-1	52	100	152
⊡ " T-2	28	50	78
+ " T-3	26	51	77



TEXT-FIGURE 8.—Survival of group A patients with lung as the primary site and having an initial neurologic function status 2.

	Censored	Deaths	Total
⊙ Treatment T-1	25	63	88
⊡ " T-2	13	33	46
+ " T-3	14	31	45



TEXT-FIGURE 9.—Survival of patients treated by treatment arms in group B.

	Censored	Deaths	Total
⊙ Treatment T-1	12	15	27
+ " T-4	7	22	29

TABLE 15.— *Percentage of patients with improved neurologic function by steroid status and intial neurologic function (group A)*[a]

Neurologic function and steroid status	Percentage of patients improved at evaluation, wk			
	1	2	3	4
Status 2				
No steroids	3.9 (102)	13.5 (89)	19.7 (71)	29.4 (68)
Steroids	10.6 (189)	20.3 (172)	27.9 (140)	32.1 (140)
Median	8.4	18.4	25.3	31.5
Status 3				
No steroids	30.4 (69)	49.1 (57)	68.2 (44)	72.5 (40)
Steroids	28.0 (200)	54.7 (170)	66.9 (136)	73.4 (143)
Median	29.4	53.4	67.8	72.8
Status 2, 3, or 4				
No steroids	15.3 (183)	29.2 (154)	40.5 (121)	46.4 (112)
Steroids	21.1 (415)	39.2 (360)	48.8 (291)	53.9 (297)
Median	19.9	36.5	46.4	51.9

[a] *Numbers in parentheses* indicate number of patients reported.

TABLE 16.—*Causes of death of group A patients, %*

Cause of death	Survival group							
	≤ 4 wk				> 4 wk			
	T-1	T-2	T-3	Total	T-1	T-2	T-3	Total
Brain metastases	45	61	47	49	39	21	40	35
Cancer at other site	33	26	23	29	36	48	38	40
Other (including unknown)	22	13	30	22	25	31	22	25
Total	100	100	100	100	100	100	100	100
No. of patients reported	73	31	30	134	193	94	90	377

or less, give poor results; the difference in survival may be due to other factors not taken into account. On the basis of the information obtained from the other 2 studies, however, it seems that the treatment regimen of 10 fractions in 2 weeks, with a delivered dose of 3,000 rads to the whole head is presently the best one for most patients with intracranial metastasis. Further work will be needed to explore the value of combining chemotherapy with radiation therapy in patients with brain metastasis. In view of the fact that long survival times have been obtained in the best, i.e., the lowest risk, groups, a trial will be instituted to see whether much higher doses over a long period of time will make a worthwhile difference for such patients.

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APPENDIX

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BRIEF PRESENTATIONS OF ON-GOING RESEARCH BY

VARIOUS COOPERATIVE GROUPS (FEBRUARY 1976)

Acute Leukemia Group B Protocol 7041

Arnold Freeman

A study of combination chemotherapy in recurrent gliomas was begun in 1970 by Acute Leukemia Group B (ALGB). At that time, only 80 patients were entered in this study, of whom 60 could be evaluated. The 25% loss was primarily due to inadequate records and inappropriate randomization. The study of recurrent gliomas, considered resistant to conventional therapy, had three arms. During its design, we debated the effectiveness of chemotherapy, and we wanted to evaluate chemotherapy versus no chemotherapy. Since most members of the group believed that it was unethical to determine the effect of chemotherapy in this manner, we elected to do a three-drug study at three dosage levels.

We gave vincristine weekly for the induction phase, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) every 4 weeks, and intrathecal methotrexate intensively during the induction phase, then monthly. The maintenance phase included BCNU, vincristine, intrathecal methotrexate, and dexamethasone. If patients were randomized among full-dose, half-dose, and quarter-dose administration, we would expect the full dose to do best, the half and quarter doses to be about 50 and 25% as effective, respectively. However, the steroids were given at the same dosage level in all three arms, i.e., 8 mg/m^2. It was proposed that the dexamethasone, acting as an anti-edema agent, be added at a constant dose to avoid masking the antitumor effect of the other three agents.

With respect to the criteria for response, we evaluated these patients by three parameters: survival; objective shrinkage in tumor size measured by scans, air studies, and arteriography [at the time the study was begun, computerized axial tomography (CAT) was not available]; and neurologic improvement. The "complete response" was disappearance of measurable tumor and a degree of neurologic improvements sufficient to return the patient to normal activity, with no sign or symptoms related to tumor. "Partial response" was a decrease in tumor size of at least 50% and an improvement in neurologic function. "Improvement" was less than partial response and "no change" was progressive disease.

The time after treatment with surgery or radiation, or both, was divided into less than 6 and more than 6 months, and the percentage response in both arms was about 60%. The overall response rate, i.e., objective neurologic improvement or tumor shrinkage or both, in all the patients was 62%. Males seemed to do slightly better than females. All patients with primary gliomas recurrent were eligible for the study, and they varied from the very young to the very old. The young did slightly better than the old, which is expected. The treatment groups included previous therapy, surgery alone, radiotherapy alone, and surgery plus radiation. Those treated with radiotherapy alone frequently had brain stem tumors or thalamic lesions that could not be explored. Again, the response rate is fairly uniform. Astrocytomas I and II had the highest response rate (80%), but only 10 patients were in this group.

Survival from diagnosis showed little difference between the astrocytoma III and IV and other tumors in this study. Please remember that the condition of many of these patients was far advanced when they were entered in this combination chemotherapy protocol. No difference was observed in survival by age, and that by sex showed little difference; although the females have a better survival than the males, the rate was not statistically significant. Survival determined from previous therapy showed no real difference, whereas that from diagnosis was about 6 or 7 months. Those patients who had a lower spinal fluid protein when they presented appeared to do slightly better, though the results were not statistically significant.

No difference was noted in survival according to the three treatment regimens, probably because few patients were in each arm, particularly when they were rerandomized according to histologic type. In addition, if the steroids did have a direct antitumor effect, they may have masked the other chemotherapeutic agents. The steroids were given at the same level, 8 mg/m^2, in all arms.

Patients who had a supratentorial lesion did better than those with an infratentorial tumor ($P = 0.05$). Some far advanced medulloblastomas and brain stem gliomas may have skewed the survival in this fashion. Perhaps the most significant result was that patients who had an objective response to chemotherapy survived significantly longer than those with no response: 9.1 months versus 3.4 months ($P = 0.001$).

In summary, the ALGB protocol 7041 showed that those patients who responded to chemotherapy had a significantly longer survival than those who did not, and this implies that chemotherapy is effective in the treatment of primary gliomas of the brain.

Greater Edmonton Cooperative Study

Bryce Weir

In this prospective study (February 1971 to April 1973), we had 41 consecutive patients with malignant astrocytoma from a catchment area of about 1 million people in northern Alberta. Patients were followed until their death. All the neurosurgeons in Edmonton contributed, so that every patient who had a grade III and IV astrocytoma was entered into the study. The same neuropathologist looked at all the tissue and made a definite diagnosis in each. One case designated as a giant cell astrocytoma was included in the grade IV astrocytoma group. In 2 patients, material from needle biopsies was insufficient for diagnosis; however, they were included. The remaining cases were all grade III or IV astrocytoma. Those who had a marked degree of giant cell changes (63%) or had a marked degree of anaplasia showed a decreased survival.

Within 2 weeks of the appropriate operation (needle biopsy, subtotal or total removal), the patients were allocated randomly into 3 treatment groups: group 1, radiation therapy; group 2, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) without radiation initially; and group 3, combination therapy. We did not wish to deprive any patient of radiation or CCNU, so when they progressed, they were crossed over to another treatment. Not all patients were crossed over as intended because some improved, some went home and then rapidly deteriorated and were not brought back soon enough to be given other therapy, and some died at home.

The first group received radiation of 4,000–4,500 rads for 5 weeks. About two-thirds of the brain was radiated with lateral opposing fields from a cobalt megavoltage unit.

We gave 130 mg CCNU/m^2 orally every 6 weeks. Half the dosage was given if the white cell count and platelets declined. Seven of 33 patients on CCNU required a delay in dosage and 8 of 33 patients, at some time or other, required a reduction in dosage.

A clinical system was established to grade response to treatment. If the patients were normal, working, and had no signs or symptoms; they were 100% at the other end of the scale, if they were hospitalized, comatose, and apparently moribund, they were in the 10% level. The average clinical grading of the survivors in the 3 groups showed a considerable spread; in all the groups, the survivors were usually well enough to be at home. We detected no striking differences in clinical grading among any of the 3 groups, which we concluded were comparable, in regard to age, male:female ratio, duration of symptoms before operation (in wk), and all the other variables including extent of removal. All other surgical, clinical, and population factors were analyzed and the groups were equal in these respects.

In days from diagnosis to crossover or progression, no significant difference was found in the median figure for the 3 groups. The combined group went 220 days before they would have been crossed over, whereas the CCNU group was under 100 days but, given the size of the samples, this still was not significant. The radiation therapy group was within the above range. The median time for survival for the combined, CCNU initial, and radiation therapy initial groups was 252, 259, and 188 days, respectively. No significant differences were noted in the means or medians for either time to progression or total survival.

Of the patients who were not crossed over, 8 were in the group who started with radiation therapy; their median survival was 122 days. The 7 who were crossed over to CCNU (after improving on radiation therapy and then worsening again and then being given CCNU) had a median survival of 357 days. In the CCNU group, 3 were not crossed over to radiation therapy, and their median survival was 304 days.

Of those who received radiation therapy initially, 7 of 15 were crossed over. Ten of 13 receiving CCNU initially were also crossed over. Of patients who had a clear-cut improvement in clinical grading after crossover (they had deteriorated following initial radiation and were then given CCNU), only 1 of 7 really improved.

In the entire province of Alberta between 1960 and 1970, we had 248 patients with grades III and IV astrocytomas with a mean survival of 6.6 months, but only 62% of those patients had received radiation therapy. In this study of 41 patients, the mean survival improved to 9.3

months, but a higher percentage of these patients were given irradiation. Thus it would be difficult to attribute this improvement to the use of CCNU alone.

This study does not provide evidence at the present time that combined therapy is better. In fact, no significant statistical differences are noted, but when analyzing the means, I believe it is probably worthwhile to use both. The mean time of stable or improved disease when radiation therapy and CCNU are used together is twice that of CCNU alone. Also, survival is about 8 weeks longer when CCNU and radiation are used than with radiation therapy alone; according to the means, overall survival is also a little better. However, we do not have enough patients to verify statistically significant differences.

Southwest Oncology Group Protocol 7404

J. Eltringham

The Southwest Group Study 7404 was initiated in late June 1974, and one of the two treatments was ended in October 1975. We attempted to evaluate the response of astrocytomas grades III and IV to CCNU versus CCNU plus procarbazine. The groups, 88 males and 39 females, had a median age of 52; the entire group ranged in age from 6 to 79 years, including 4 children.

The only previous treatment allowed before randomization was surgery with or without any steroid. All patients received 6,000 rads in 7 weeks and 10 mg dexamethasone/m^2, which usually tapered off in 5 to 6 weeks. CCNU was given 48 hours before radiation at a dose of 100 mg/m^2. An escalation was built into the protocol: If patients tolerated the first dose well, most went on to 130 mg/m^2, with appropriate adjustments.

On the other limb, we gave radiation therapy, dexamethasone, and CCNU; 75 mg/m^2 for the first dose, with 15-mg steps for escalation or deescalation. The patients received procarbazine alone, but only 2 of the 33 patients who could be evaluated on that treatment arm were crossed over.

Our criteria for grading response were as follows: "complete response," "partial response," "no change," or "increasing disease." Complete represented total disappearance of measurable central nervous system tumor, a normal neurologic and/or psychometric examination in the absence of symptoms. Partial improvement represented decreasing size, improving neurologic examination, or improving symptoms. No change was a steady state or no evidence of progressive disease. Increasing disease, again, was obvious increase in size, deterioration neurologically or psychometrically, and worsening symptoms due to a tumor.

The procarbazine and CCNU limb was closed in October 1975 because we encountered three episodes of significant dermatitis. Two of the three patients had been taking dilantin. The third, who had the most severe reaction, sloughed most of her skin and had to be treated as a burn patient; she had responded to treatment but relapsed and died as the tumor progressed.

At present, the group receiving CCNU alone contains 33 patients who can be evaluated; most of those who cannot be evaluated are in the early stages of their courses of treatment. In the combined limb, 32 of 60 patients can be evaluated; again, most of those scored as "not suitable for evaluation" are in the early phase of treatment.

After approximately 1 year, we had 40% complete and partial responders in the group given CCNU. Fourteen of 33 (42%) patients were stable at approximately 1 year. Six patients showed progressive disease, 5 of whom died. Two more who were crossed over also died shortly afterward.

As noted earlier, the CCNU plus procarbazine group contained 32 patients who could be evaluated. The numbers of complete and partial remissions were 2 and 7, respectively, or 28%; as of the last analysis, they had 228 days without recurrence. The no change group with 15 patients (47%) included 1 who survived 459 days as of February 1976. The disease progressed in 8 patients, and of these 4 died rapidly.

The principal toxicity we encountered in the combined CCNU plus procarbazine group was manifested as rashes. In addition, 11 patients (approximately 16%) have had pulmonary emboli, which were fatal in 5. These emboli occurred while the patients were receiving radiation therapy and had either just been tapered off or were still receiving dexamethasone. Another patient developed thrombophlebitis and several more had pneumonia that might have represented pulmonary emboli. Most of these patients were active, had performance ratings in the range of 70–80, and varied in age from 28 to 69 years.

At the last review, we concluded that the CCNU limb had a slightly higher response rate, 40 versus 28%. The numbers are small but we may see more significant differences as data accumulate on some of the newer entries. Numerous patients in both limbs were stable, 42 and 47% after a year in the study. Hematologic toxicity was comparable in both limbs, but more patients in the CCNU and procarbazine group refused further treatment.

European Organization for Research on Treatment of Cancer Brain Tumor Group Studies 26741 and 26742

J. Hildebrand

The European Brain Tumor Group is studying the effect of CCNU in patients with malignant glioma, using three criteria: the free interval, rate and length of objective remission, and total survival. In effect, we are running two parallel trials within the same study.

To be admitted to the first study, the patients must be over 16, the diagnosis of malignant glioma must be proved by tissue pathology, the operation had to be performed 3 weeks before admission, and the expected survival should be more than 8 weeks. Patients admitted to the first study met two additional criteria. First, they did not require dexamethasone. We stopped dexamethasone within 1 week after operation, waited 2 weeks, and if we believed that the patient no longer needed dexamethasone, he was admitted to the study. The second criterion was that the neurologic examination was almost normal. Patients admitted to the second study could receive dexamethasone if necessary. Their neurologic examination was usually worse and they had frequent minor abnormalities, although the expected survival also was more than 8 weeks.

So far, we have admitted 100 patients into the first study, and the results I shall report are from the first review of 81 patients in that group. We asked the following questions: In this selected group of patients, could we measure the free interval between operation and relapse? If so, does CCNU prolong the free interval? In patients who did not receive chemotherapy before relapse, we sought to determine the rate and the length of objective remissions due to CCNU and dexamethasone. Objective remissions induced by dexamethasone and CCNU were defined as an objective improvement of neurologic examination that would last at least 4 or 6 weeks *after* complete discontinuation of dexamethasone. This criterion assumes that the effect of steroids is sustained only as long as they are maintained, and that the improvement continues 6 weeks after complete discontinuation if the effect is due to CCNU.

In the second study, we wanted to measure only the effect of CCNU on total survival. We concluded that we would measure the free interval in this selective group of patients if we followed them every 3 weeks. Any suspected recurrence then would be confirmed within 2 weeks by neurologic examination, EEG, scan, and arteriography.

CCNU does not seem to affect the duration of the free interval, the median of which is about 34 weeks in both groups. I should stress that dexamethasone was never given to these patients during the free interval. The amount of CCNU given was 106 mg/m^2 every 6 weeks instead of the scheduled 130, chiefly because of hematologic toxicity and, in a few cases, digestive intolerance.

Does the CCNU induce objective remission in patients who did not receive it immediately after the operation? The answer appears to be "yes." Thus far, we evaluated only 16 of these patients; 4 had an objective remission lasting from more than 24 to 53 weeks.

Finally, we compared the total survival of patients who received CCNU early, i.e., immediately after operation, with those who received CCNU only after recurrence. Apparently, survival is slightly but significantly longer when CCNU is given late. Since the free interval is the same in both groups, we concluded that patients survive longer after recurrence if they do not receive CCNU immediately after operation.

The administration of dexamethasone was not mandatory but was allowed after operation in the second study. Here we were trying to determine whether CCNU could prolong survival. To date, only 20 patients have been included, chiefly because we have to keep controlled and treated patients under similar conditions to measure survival accurately. In other words, they should be hospitalized, at least when their condition deteriorates. In these patients, supportive care may be as important as chemotherapy, especially if the latter is not extremely effective. Despite our care, only 14 patients of the 20 fulfilled this criterion; the 6 who died elsewhere were distributed equally between the two treatment arms.

Dexamethasone administration was allowed, and we asked the participants to report how much the patients actually received, but they did not. In Europe, we have the impression that people who are strongly dependent on dexamethasone take it for all kinds of symptoms; headaches, indigestion, and even gastric pain. Although we tried, we could never determine how much dexamethasone these people were taking. Nevertheless, the group who received CCNU survived longer and, in this small group, this longer survival was significant at the 0.05 level.

In conclusion, we believe that CCNU affects the survival and that it may induce objective remission in 20% of the patients. As far as the free interval in a selected group of patients is concerned, we believe that CCNU given immediately after operation confers no benefit and may even do harm. I must emphasize that all these conclusions concern CCNU alone, and that the mean dosage was 106 mg/m^2 and not the scheduled 130 mg/m^2.

Southwest Oncology Group Study 74-09 in Children

J. van Eys

This study was designed to determine if adjuvant chemotherapy (vincristine and intrathecal methotrexate) can reduce morbidity and mortality in children with ependymoblastoma and medulloblastoma. The second objective was to see if the staff of the pediatric section of the Southwest Oncology Group could develop workable criteria for response.

The study design was as follows: operation followed by radiotherapy, then randomization to a "chemotherapy" and "no chemotherapy" arm. Because of the prolonged entry of patients into the study, reporting at this date is far from complete. All patients in the pediatric age group were eligible, and many institutions in the Southwest allow those up to age 21. So far, the oldest is 16 years, and the youngest is 1 year. All had surgical tissue diagnosis that will be reviewed later by the pathologist of record. The patients had no prior treatment except local corticoids; they were stratified by age and, if necessary, by the presence of a shunt. No patient has yet had a shunt.

Radiotherapy was standardized. For medulloblastoma, the dose was 3,500 rads for children less than 3 and 4,000 for children over 3 years. The spinal cord dose was 2,500 and 3,000 rads, respectively, with a posterior fossa "boost." For ependymoblastoma, the dose was 3,500 rads for both age groups and 2,500 and 3,000 rads for the spinal cord.

Initially, the chemotherapy dosage was 2 mg vincristine/m^2, with a maximum dose of 2 mg; 15 mg methotrexate/m^2, with no maximum dose, and 15 mg hydrocortisone/m^2, which is added because data from the Southwest Oncology Group seemed to indicate that this decreases meningeal irritation; no maximum dose was designated.

The total number of patients entered to date is 17, 2 of whom were lost to follow-up.

Failures are defined as either death or progressive disease. The latter is established by two of the following four criteria: increased 1) measurable lesions, 2) symptoms, 3) intracranial pressure, and 4) spinal tumor cells on two sequential spinal fluid examinations at least 2 weeks apart.

The most important question with which we were concerned is: Is this treatment feasible at the present time? Marrow suppression occurred in both groups as expected, and we had one death due to sepsis secondary to marrow depression. One patient had severe but reversible toxicity from vincristine, which was discontinued. One child died with progressive central nervous system degeneration about 2 months after completion of radiotherapy, but no autopsy was obtained, so the tissue diagnosis in this case is unknown. The study is continuing.

Western Cooperative Oncology Group Studies 807, 808

L. Irwin

Hydroxyurea, a polar nonlyophilic drug, is one of a small number of cytotoxic agents that pass into the cerebral spinal fluid (CSF) after systemic administration. In addition, in animal studies, hydroxyurea causes synchronization of cells in the late G_1-phase and thus may potentiate radiotherapy effects. Studies in our laboratories show that small divided doses of this agent will provide significant levels of drug in the CSF. We measured CSF levels in 6 patients after administration of 10 mg hydroxyurea/kg body weight, given at 6-hour intervals for 3 days. The drug enters the CSF in levels comparable to or higher than the level achieved by continuous iv administration or a single large oral dose, i.e., 80 mg/kg body weight.

We developed 2 studies to explore the influence of hydroxyurea added to radiation therapy in prolonging survival of patients with malignant cerebral gliomas. In the first (No. 807), patients were randomized without stratification in a double-blind fashion to receive either hydroxyurea or placebo in identical capsules at 10 mg/kg at 6-hour intervals, every other day. This started 1 week before and continued throughout the course of radiation therapy, at the end of which time the drug was discontinued. Radiation therapy was administered with cobalt teletherapy to a dose of 4,800 rads to whole brain, with a 2,400-rad boost to the tumor bed. This was given for 11 weeks with two 1-week splits. The nominal equivalent dose of this program to the tumor bed is approximately 1,900 rads.

During this study, we noted that some patients in regimen A worsened and died precipitously. At autopsy, they had extensive tumor necrosis that seemed to be greater than one would expect from radiation therapy alone. When the code was broken, we found that regimen A patients were receiving hydroxyurea and not placebo. The study was then modified to include a second operation done 2–4 weeks after radiation therapy was completed to remove necrotic tumor.

Twenty-eight patients were entered in study 807; 13 were randomized to receive hydroxyurea and 15 to receive placebos. One patient randomized to hydroxyurea died of a pulmonary embolus before receiving any therapy and was not evaluated.

Thirty-two patients were entered in the second study (No. 808); 16 were randomized to the hydroxyurea arm and 16 to the placebo arm. One patient in each arm did not receive the drug because each withdrew from the study; thus they were not evaluated. All patients for evaluation in both studies are included in survival analysis. Five of 27 patients treated with hydroxyurea plus radiation therapy were alive at the last analysis as were 2 of 30 in the placebo arm.

The ratio of male to female in the hydroxyurea and placebo arms was 2.38 and 1.50, respectively. The median age for the hydroxyurea group was 53 years, with a range of 21–67, and for the placebo arm, 56, with a range of 19–66. The median period from symptoms to operation was 3 months, with a range of 2 weeks to 48 months in the hydroxyurea arm, and 2 months in the placebo group, with a range of 2 weeks to 37 months. The median period from operation to the start of therapy was 3 and 4 weeks for the hydroxyurea and the placebo arms, respectively.

Therapy with hydroxyurea or placebo was interrupted if the white cell count was less than 2,500 or the platelet count was less than 60,000. Toxicity was mild, and at no time was it necessary to remove a patient from study because of toxicity. Patients receiving placebos had evidence of hematologic toxicity, which was attributed to distant effects of local radiation.

All histologic material was reviewed retrospectively by our referee neuropathologist, Dr. Richard Davis. Two patients were entered who had oligodendroglioma. Eighteen patients are classified as anaplastic astrocytomas, and include gemistocytic, fibulary, and protoplasmic astrocytomas. There were 37 patients with glioblastoma.

As one might expect, patients with anaplastic astrocytoma had longer survivals than those with glioblastoma. The effect of hydroxyurea plus radiotherapy in prolonging survival was most notable in the anaplastic astrocytoma group. The actual median for the hydroxyurea group was 42 months versus 10.8 months for the placebo arm. Differences in survival also were demonstrated for patients with glioblastoma; the median for hydroxyurea was 10.4 months versus 7.8 months for the placebo arm.

With respect to location of tumor, those with frontal lobe lesions had a slightly better survival than those with temporal and parietal lesions. This difference was more pronounced but not significantly so when hydroxyurea was used.

In the 27 patients in the first part of our study, the median survival for the hydroxyurea group (calculated from the start of chemotherapy) was 11 months versus 9 months for those given placebos. This difference was not statistically significant.

Of the 30 patients in the second series (which included a second operation), those receiving hydroxyurea had a median survival of 14 months versus 10 months for the placebo group. Again, the difference was not statistically significant due to the small number of subjects.

When we combined the patients in the 2 parts of the study, 27 patients were treated with hydroxyurea plus radiation therapy and 30 the placebo plus radiation therapy. The median survival of the hydroxyurea group is 14 months versus 9 months for the group receiving placebo plus radiation therapy. This difference is significant at the 5% level.

To study the influence of the second operation on survival, we combined the data of patients who had received hydroxyurea plus radiation therapy in the 2 studies and divided them into those of patients who had a second operation and those without. The second operation does not appear to prolong overall survival.

To summarize, hydroxyurea added to initial operation plus radiation therapy apparently is effective in this group of patients with malignant cerebral gliomas. This drug should be combined with other agents and, currently, we are considering hydroxyurea plus radiotherapy plus BCNU versus a control arm of BCNU and radiotherapy. Finally, a second operation does not contribute significantly to increased survival and, in fact, may exert an unfavorable influence.

Radiation Therapy Oncology Group and the Eastern Cooperative Oncology Group Study of Patients With Malignant Glioma

S. Kramer

Our study is in progress and therefore we can give you only preliminary findings. The study chairman for the Eastern Cooperative Oncology Group is Dr. John Holton and for the Radiation Therapy Oncology Group, Dr. Chu Chang; our bionutritionists are Dr. Zellen and Dr. David Schoenfeld from Buffalo.

We believed that combined management of malignant gliomas deserved further exploration and hence began at the point where the Brain Tumor Study Group concluded. We take all patients with malignant gliomas and stratify them by histology, extent of surgery, and institution, then randomize into four branches. Six thousand rads in 6–7 weeks to the whole brain provides our controlled base line. To that we added BCNU for the record arm. Then to the same doses as the Brain Tumor Study Group, we are adding 1,000 rads in 1–2 weeks, giving us a total dose in the involved portion of the brain of 7,000 rads and about 8.5 weeks. All treatments are to the whole brain. We then add methyl-1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU) and dimethyltriazenoimidazole carboxamide (DTIC) as the fourth branch. In addition, we are conducting a small pilot study to see whether we can use radiation sensitizers given intra-arterially in unilateral lesions, which are uncommon in these highly malignant gliomas.

The study was begun in July 1974 and, initially, we had considerable toxicity in the two chemotherapy arms. Somehow in the BCNU study, after the patient received the initial 80 mg/m^2 on 3 consecutive days, the subsequent courses were given, in some instances, in a single dose rather than divided by 3. The response progresses with the number of cycles, so that almost one-third of the patients who had full doses developed severe toxicity. We now propose returning to the original schema of 3 divided daily doses as before.

Initially with these doses, we encountered considerable toxicity with Me-CCNU and DTIC, and all agreed that the doses of CCNU be reduced to 125 mg/m^2 and those of DTIC to 150 mg.

With this new schedule, we have seen only one severe case of toxicity in 8 patients. In these patients, the diagnosis was established at operation. We accept minimal and maximum surgery, and this will be stratified retrospectively. We also allow an option: not every participating institution has to take all four options, but they must take at least two. We now have registered 181 patients and, so far, the options have evened out well.

I am reporting here on the first 141 patients registered. With respect to histologic grading, we have referred to grades III and IV astrocytoma and found them in about equal numbers. No grade was stated in another large group. We believe that in classifying malignant gliomas, a distinction has to be made between the less malignant and the more highly malignant tumors because the survival of these patients differs on the basis of histology. I am not recommending that they be graded according to the Kernohan scheme, but it is important that we agree on some kind of stratification by degree of malignancy and by histologic pattern because these clearly have predictive value.

To date we have a survival curve with a median of 31 weeks. As they are divided into grade of malignancy, a clear separation begins to evolve between grade III and IV lesions. This separation probably would be more marked except that the unclassified gliomas (about 20% of the whole group) have been included in the grade IV's. If those were excluded, there would be a greater distinction between these two classes. Even so, the median survival of the grade III's is 36 weeks and of the grade IV's 29 weeks, and that, in a relatively short survival, is a marked difference. This emphasizes the point I made earlier that some kind of division according to the degree of malignancy is mandatory if the results are going to be meaningful.

The early survival curves show that some discrepancy is beginning at the 50% survival, but to date, none of the four treatment arms is sufficiently different to oblige us to make changes in this particular protocol.

In summary, we are adding about 80 to 90 glioma patients to our study and to the combined intergroup study between the Eastern Cooperative and Radiation Therapy Oncology Groups. We now have achieved an acceptable level of toxicity, and we propose to accumulate more patients before reporting on survivals.

DISCUSSION VI

M. Walker: Dr. Audrey Evans will report on the combined study being done by the RTOG and the Children's Group.

A. Evans: The Children's Cancer Study Group and RTOG Study for children with medulloblastoma was activated in June 1975, so I will describe the study design only. The purpose is to determine the effect of local recurrence or metastasis and if chemotherapy would increase the time to recurrence and the survival rates. Secondary objectives are to determine the toxicity of the chemotherapy planned and to examine the effect of various prognostic factors. Randomization with radiation therapy plus or minus chemotherapy was done by phone to the central office 3 weeks after surgery. The patients were stratified according to stage of disease and extent of metastasis. Age and sex also were stratified. Eligible patients were those with histologically proved medulloblastoma, and who were between 2 and 16 years of age, within 3 weeks of operation, and stable. In the Children's Group, ependymoblastomas also were included. Patients who received chemotherapy other than corticosteriods, had had a bacterial meningitis, or had extracranial metastasis were ineligible.

All patients received X-ray therapy, and the group randomized to the chemotherapy arm received vincristine simultaneously, then had prednisone, vincristine, and CCNU in 6-week cycles for a year.

A second phase was established in the hope that patients would be kept on study for as long as possible. Those who failed in the first phase, i.e., within 1 year from their irradiation, received chemotherapy alone. If they failed more than a year after radiation, they received radiation and chemotherapy. The patients who had been in the chemotherapy arm then received procarbazine alone; those who had had no chemotherapy received vincristine, CCNU, and procarbazine.

X-Ray therapy to the CNS axis consisted of 3,500–4,000 rads at the rate of about 800–1,000 rads/week. An additional boost of 5,000 brought the exposure of the posterior fossa to 5,000 or 5,500 rads. Chemotherapy consisted of 1.5 mg vincristine/m^2 for the 8 weeks of radiation therapy, followed by 1 month's rest, and then cycles of 3 doses of vincristine, 14 days of prednisone, and 1 oral dose of 100 mg CCNU/m^2.

The study is designed to measure the disease-free interval and time to recurrence. No attempt is made to measure the response to chemotherapy, e.g., in the reduction of the tumor size. Definition of recurrence is difficult, but the study committee agreed that any recurrence had to be documented and the 3 investigators (surgeon, radiation therapist, and chemotherapist) must concur that recurrence or progressive disease has indeed taken place.

In the first 6 months of this study, 26 patients have been entered, and at the last analysis, 14 patients, 7 in each arm, were available for analysis of disease status and toxicity. As yet, no patient in either arm has failed. Significant toxicity has occurred in both groups during the initial radiation therapy. One child who was not receiving vincristine developed a platelet count of 10,000. Another developed severe herpes zoster, and a third has had a *Pneumocystis carinii* pneumonia, which is an unusual accompaniment to X-ray therapy alone. A group in Los Angeles County Hospital, who has been using this protocol but has not entered patients in this study, has also encountered significant toxicity. It is early in the course of study, and probably what we are seeing is the known toxicity that accompanies radiation therapy of the CNS axis.

Walker: All the data you heard today are preliminary. Remember, they are going to be changing continuously. We should not expect a major change in the treatment of this disease or expect to make great strides with current chemotherapy. The precision, control, and discipline shown in these studies are necessary just to define small differences and to put us in a position to capitalize on them.

F. Hochberg: In view of the apparently conflicting data concerning the synergistic effect of CCNU and radiotherapy in primary astrocytomas and a Mayo Clinic study that suggested that, in the double-blind situation, CCNU was not efficacious, would you comment on the synergistic value of CCNU in the treatment of these tumors?

J. Hildebrand: I would disagree with the conclusion that CCNU is ineffective. Any judgment on its value depends on the parameter being used to measure effect. A given drug may induce objective remissions, yet not prolong total survival. Earlier studies have shown that a drug must give 70–80% objective remissions to prolong life significantly. Dramatic objective responses, which occur in about 20% of the patients, do not lengthen survival significantly or affect the free interval or the total survival. These are different parameters. Also, one should establish with certainty how much of the drug is given, not only the scheduled dosage but also the actual dosage given. At the Mayo Clinic, 130 mg CCNU/m^2 was given every 8 weeks, which is a low dosage. This was the scheduled dosage, but how much CCNU was actually given? I agree that CCNU does not constitute a major breakthrough in the treatment of brain tumors, but there is no major disagreement concerning the results.

Walker: This shows the difficulty in conducting clinical trials and the necessity for precise and comparable data. Parenthetically, they must be comparable in terms of patient population and also in terms of the therapy patients actually receive.

B. Weir: Our data show that patients who received CCNU plus the radiation therapy had about 2 more months of stable or improving disease over those who had radiation therapy alone. We do not use it in all patients now, since we believe it has only a marginal effect.

C. Wilson: Dr. Kramer stated that he saw more toxicity when he switched from the 3 daily doses of BCNU to the single dose. How much was given with the single dose?

S. Kramer: We were giving the same total dose and that is clearly a mistake. I think we have to reduce the dose or go back to Dr. Wilson's original schedule, which seems to be safer and possibly more effective.

Wilson: A dose comparable to the three times 80 mg is probably 180–200 mg; 180 mg would probably be safer. In redesigning our recent protocols, we went back to the single dose, based on laboratory evidence and on the desire to save hospital time and reduce morbidity with shorter courses of radiation therapy in patients with metastatic disease. If one can give chemotherapy in a single dose, even if it is no better, one is considerably ahead. We believe, on the basis of some kinetic work, that a single dose will be a little better.

G. D'Angio: Study design is most important when one is looking for small differences and various kinds of objective responses. In making comparisons between studies, are we all using the same base lines? This is particularly true in protocols that involve differences in the timing of the total course of therapy or a secondary maneuver of some kind. The result can be dated from the completion or from the initiation of therapy, but either involves built-in biases. In Dr. Kramer's study, 1,000 rads was given in 1 shot versus 4 in 4. How do you take care of that timing? Obviously, patients are going to die between the first day of treatment and the 4th week. How do you handle that kind of problem?

Kramer: Dr. D'Angio's point about timing is extremely important. In the RTOG, all our survival data are calculated from the first day of treatment. I believe it is highly desirable that all groups should measure survival from the same time. The first day of treatment is more convenient when treatment schedules are of different lengths. Second, when so many studies have been reported and some with relatively marginal differences, it is important to gather the information in such a way that, when different groups have almost the same results but not quite the same study, the comparability becomes much less. This is why we copied the two arms of the BTSG, so that presumably we ultimately would be able to put these groups together and see whether any differences are due to factors other than treatment parameters. If people cannot agree to do the same study, they should at least agree to have common arms, because it gives us the opportunity to compare the studies.

Walker: One of the most important objectives of this conference was to delineate some of these base-line factors, whatever they may be. I am not saying that any one is right or wrong, but we may be able to get some common parameters.

Wilson: The Edmonton study is a perfect example of a situation in which we are looking at small differences. One could say to the statistician, "We would be happy with a 20% improvement with chemotherapy over radiation therapy. We would like to do this with about an 80% probability of not missing the opportunity if it exists. How many patients do we need in each group?" If the Edmonton study was two or three times larger, it would clearly have reached statistical significance, but the numbers were too small. At this point, we were happy to see 20 and 30% increases in survival. In designing such a study, one would have to go to the statisticians and say, "How many patients must participate?" or, in this instance, say that the trend is there, even though the statistics do not support it.

Walker: My recollection is that about 300 patients are needed in each group to detect a 20% response with an 80% probability. Clearly, this is a difficult number to accrue in any such study, and it prompts the question as to what percentage response we should insist is an acceptable change. I do not think a 20% improvement in survival is worth obtaining.

L. Marshall: I would like to make a plea for the stringent control of corticosteroids and agreement on a specific steroid and a specific duration of treatment. In any of our treatment regimens, the data are going to be contaminated unless we control the use of steroids; the dose, the duration, and how and when they are administered.

Walker: I agree. One of the major objectives in the next study by the BTSG will be the oncolytic effect of corticosteroid as it might be separated from its edema-controlling effect. To provide an answer to this question, one of the major therapeutic arms will be the use of high-dose corticosteroid. This will be a satisfactory study because it is a "no lose" one. If corticosteroids do not prolong survival, we can use them with a certain freedom and make our patients comfortable, as well as the referring physicians and ourselves. On the other hand, if it proves to have an oncolytic effect, we have added another drug to our armamentarium.

Evans: We put prednisone in the chemotherapy arm of the medulloblastoma study because it was difficult to control corticosteroids. People often start them at random, e.g., in the "low" after a course of chemotherapy. We had the uncomfortable feeling that physicians would add dexamethasone for a while and that corticosteroids may have an oncolytic effect. However, we hoped that we could institute control for the variation in corticosteroid usage if all patients receiving chemotherapy also received corticosteroids in a 14-day course of treatment that is then tapered off in 1 week.

Weir: Of our 8 autopsied cases, 3 had large pulmonary emboli, which presumably caused death. Although all had received CCNU, we thought the emboli resulted because the patients had been hemiplegic and bedridden. All had also been on steroids.

Walker: The Southwest Group has had some sick patients and a large number of pulmonary emboli were observed. I believe 8 patients had pulmonary emboli, but no one knows what relationship this might have to chemotherapy. To my knowledge, no other investigators using these drugs have seen an unusually large number of emboli. Apparently, pulmonary embolus is not uncommon in the bedridden patient who has glioblastoma.

Marshall: We have looked at 78 patients with glioblastomas, all of whom were receiving high-dose dexamethasone; 29 had needle biopsies, and the remainder had had craniotomies for glioblastoma. The incidence of pulmonary embolus in that group as a whole is about three times higher than in patients with other intracranial disease on a neurosurgical service. Steroids may have an influence, but the major factor seems to be the clinical status of the patient. These patients are in the hospital, and pulmonary embolus is more easily and more frequently identified in that setting. Our experience suggests that in patients with malignant gliomas, a significantly higher incidence of pulmonary embolus and other hypercoagulable events, such as thrombophlebitis, occurs. We have done some preliminary studies on clotting factors and have not identified any definite difference, although there is a suggestion that platelet adhesiveness may be increased in such patients.

Walker: Did you say that pulmonary emboli are three and one-half times more frequent among patients with gliomas as compared with all other patients on a general neurosurgical service?

Marshall: When pulmonary embolus was a factor leading to death, that frequency appears to be correct; we do not have enough information, which is the reason it has not been published. Also, we have been searching diligently for some kind of coagulation index that will allow us to identify the factor responsible. This predisposition has been suggested before in a patient with head injury, because of the release of brain thromboplastin in a patient with this definite injury. Perhaps a similar phenomenon occurs in patients who have glioblastoma. In any event, the most significant factor seems to be the clinical status of the patient; i.e., whether he is hemaplegic or bedridden. There is a strong suggestion that glioma patients seem to be at greater risk, and we are studying the association.

J. Eltringham: With regard to sex difference, in the Southwest Study 11 patients were seen with pulmonary emboli; 9 were males, with 3 fatalities; 2 were females and both died. Most of the patients were reasonably active and none was bedridden except one who had been recently hospitalized, discharged, and who returned several days later with a fatal pulmonary embolus.

Walker: Obviously, a factor of great concern to all of us has been identified, although we do not know whether it is of statistical significance. I ask each of the study chairmen to add to the list of factors they are following on their patients the specific question of the frequency of pulmonary emboli in those who are receiving steroids and nitrosoureas. If the study chairmen will add this factor and will ask their groups to collect the information, we will know we are talking with precision in a couple of years when we meet again.

D. Lane: Dr. Kramer's data showed that by the 8th week, no difference was noted between the group receiving steroid therapy and those who were not. In the study of pulmonary emboli that has been proposed, we should limit the amount of steroids, particularly for chemotherapy studies, and if steroid therapy is reinstituted, e.g., for recurrence, we will be dealing with the same groups.

Kramer: I find it difficult to put a strict limit on steroids, especially in clinical situations in which the brain tumor patient suddenly becomes worse. Sometimes these events are unrelated to the tumor because a number of stressful situations do make these patients worse. Under such circumstances, can you stop these patients from being given steroids? If you can, that is fine. Also, is there a level of steroids, either in duration or dose, that would be a "no steroid" level?

Walker: Our experience in the BTSG suggests that we cannot effectively control the use of steroids; physicians are going to use them because they have such dramatic effect on patients. However, we can collect the data and form valid opinions on their relationship to such events as pulmonary emboli. Dr. Hildebrand noted that his patients probably were taking a little dexamethasone when they were feeling poor. In our studies in Baltimore, we found that when patients begin to get into trouble, they will take some tablets from a bottle of leftover steroids.

J. Horton: One of the principal reasons for having a conference like this is to translate research findings into suggestions for clinical practice. The results presented from the BTSG are selected from a specific population of patients considerably smaller than the original randomized group. We should like to get an idea of the effect of a treatment policy rather than the effects on a specific series of patients. When you considered all the patients entered in the study, did you note the differences in median survival from the various treatment groups or were they present only in those adequately treated?

Walker: The trends are there, but they are not clear-cut. There are more variables and more "noise" in the system. I did not present all the data, but all three types of survival curves look similar. True differences do not come through as well in the randomized population because low-grade gliomas are mixed until they are culled out by neuropathologic evaluation. In addition, other patients have been placed on other forms of chemotherapy, and they have to be removed before the population is really uniform. An adequately treated group is studied specifically to intensify the natural differences when we are looking for small changes in the value of these forms of treatment.

Evans: In those data, you could take the patients who were legitimately on study and cull the ones who did not belong, e.g., those with a low-grade glioma. Then you could look at the results of patients who were available for study and who were randomized. It would be interesting to know, if you had so many early fatalities in each group, that your difference disappeared completely.

Walker: The trend holds true for both the adequately treated group and those who have, in Dr. Kramer's terms, malignant gliomas, or glioblastoma multiforme, grade IV (if you pull those out as a separate category). We can take all these various cuts at various levels and the trends are consistent throughout the study, but they are small trends.

A. Farca: Dr. Hildebrand, can you provide any direct assessment of what the radiation with or without chemotherapy has done to the brain tumors, particularly from evidence obtained at second-look operations. Does the tumor really shrink and almost disappear except for the necrosis due to therapy?

Hildebrand: You are referring to the patients who have objective remissions. The only evidence we have for tumor shrinkage is that the remission induced by dexamethasone and CCNU together would last for weeks and months after dexamethasone was stopped. We have clinical evidence and confirmation from EEG and brain scan that the tumor is smaller, but we have never done a second operation to establish this. May I add that when we speak about steroids, we usually speak about the favorable effect of these drugs. However, people who are on steroids for a long time often develop proximal weakness of the lower limbs, and because we use the Karnovsky index to assess the effect of treatment, this proximal weakness in bedridden patients especially may greatly prejudice our evaluation.

Walker: Again, when we receive a patient's Karnovsky score, we take into account whether he has been receiving a steroid.

D'Angio: I would like to discuss the lymphopenia that follows radiation therapy. In your study, Dr. Walker, did you include this as one of the variables? Did you have an opportunity to compare the patients with and without lymphopenia who had been given radiation therapy?

Walker: I am embarrassed to say that we did not do blood studies; our investigators felt that this would be an undue expense for the patient. I disagree, but we do not have that information.

Kramer: I think the marrow depression we see is almost exclusively due to irradiation of the spinal cord. In your group, Dr. Walker, I believe the spine was not irradiated. We do routine counts on all our patients, and it is most unusual to see a major depression when only the skull is radiated. Those of us who treat medulloblastomas using a kind of "overlap" schedule encounter major myeloid depressions only when treatment to the spinal cord starts. The brain is radiated only for the first 3 weeks, and then irradiation of the spinal cord is started; the two are treated concurrently for a couple of weeks and then the spinal cord is finished.

D'Angio: I believe you simply catch a certain amount of the circulating blood pool and thus get a direct lymphocytic effect, as you do when irradiating the mediastinum or the chest wall. I would expect to see an absolute lymphopenia.

Walker: I find it hard to believe that daily radiotherapy would not affect the brain, which is receiving about one-quarter of the cardiac output. You are irradiating the total blood volume daily for 6 weeks.

I would like to return to the idea of a second-look operation. In my view, you do not and cannot do "second-look" operations until the patient is in trouble again, until you sense that you have a tumor that is operable and is in a favorable location. You intend doing a "debulking" procedure, which you can do within the closed cranial vault and thereby buy more time. When you operate again on these patients, you are admitting that your prior therapy is a failure. That is not entirely true, however, because you may have altered the growth curve of the tumor, although you may not have stopped it completely. I would appreciate other thoughts on second-look operations.

R. Hittle: We looked at more than 700 patients who had acute lymphocytic leukemia, with varying amounts of radiation therapy over an extended field, craniospinal, head with intrathecal methotrexate, and methotrexate alone in relationship to associated infectious processes. On study, there is a frequency curve that begins at about the 30th day and extends through the 80th day, and the 2 groups are cleanly demarcated. Twenty-six patients in the first two limbs (those with extended irradiation) had infectious diseases of bacterial origin associated with a granulocytopenia, and 20 patients who had head irradiation, or intrathecal methotrexate alone or with the head irradiation had atypical infections. The granulocytopenias were not considered unless the patients' lives were in danger. In the second group, we had one crossover to irradiation, and in the bacterial infection, we had one near-death episode in the lower groups. Among the patients with head irradiation and intrathecal methotrexate or the drug alone, lymphopenia was the prominent feature, and this occurred at a specific time, i.e., between the 30th and the 80th day on protocol. This is an important matter about which we need data for other brain tumors.

Marshall: In our experience with second-look surgery for other disorders, the incidence of infection and failure of wound healing has been extraordinarily high. Irradiated patients who had to be operated on for other reasons also had wounds that healed poorly. A significantly increased incidence of infection is seen, and this is a general experience of most neurosurgeons who have to go through an old craniotomy site within 6 months or a year. The infection rate has been extraordinarily high in such wounds. Anyone contemplating these procedures should be forewarned: patients, who have some degree of immunosuppression and who may have received significant irradiation to their heads, may represent serious trouble.

Weir: The classical series contain enough information about grades III and IV astrocytoma to dissuade anyone from adopting the second look as a policy. Survival after second operations for high-grade astrocytomas is only a few weeks. The CAT scanner now provides an accurate assessment of what is happening to the tumor and to local edema, and we can fit this information with the clinical picture so that an operation on that ground can no longer be justified.

J. Hekmatpanah: I would like to advocate a "second management" rather than a second-look procedure for some of these patients. We have seen several cases in which the first surgical removal was inadequate, and the second operation permitted a wide resection. At times, we encountered a cyst collection, and puncturing or removing this collection has prolonged the patient's life by 2 or 3 months. Occasionally, a shunt procedure has helped some patients who developed hydrocephalus because of high protein or a secondary adhesion. At times, the physician should obtain another opinion concerning a second surgical procedure.

M. S. Mahaley: There are times when you do want to take a second look, but these are infrequent. Some of our more rewarding clinical results are rare instances when we planned a second look at the time of the first operation because of the location of the tumor and its relationship to major draining veins on the surface of the brain that precluded radical removal. At the first operation we found it was an anaplastic glioma and then, at a later date, we went back and did a more thorough resection. This is indicated rarely, but for the type of case the BTSG would see, it is unlikely that the patient will benefit or that the surgeon will learn much from a second resection. Also, we would be gravely concerned about wound healing and infection.

Lymphopenia in patients with glioblastoma of the brain has to be taken in perspective. What are your base-line values? Thirty-six patients we studied before surgery or any other treatment had a lymphopenia, with an average of 1,400 cells as compared with matched controls. Lymphopenia may be even more significant in these patients than in others. Our patients with meningioma and those with anaplastic gliomas that are not clearly glioblastomas do not have this degree of lymphopenia. It is not just the quantitative count of these lymphocytes; we should ask what population of lymphocytes is specifically reduced. Are these T or B cells? Does this have an effect on the patient's own immune mechanisms? Finally, even though the lymphocyte counts may be within normal limits, do these cells possess the functional capacities they had before therapy was instituted?

Walker: You gave your absolute count for your patients as 1,400. What was the control?

Mahaley: It was 2,400. These are not total white cells; they are lymphocytes.

W. Bingham: If a second-look operation is considered, it should be considered at the time of the first procedure because so many things can go wrong the second time, including infection and additional technical difficulties. Supposedly, one of the reasons for doing second-look surgery is to do the job better the second time. Unfortunately in brain surgery, the landmarks are often so distorted from the tumor and from the previous operation it is often difficult to know exactly where one is. Second-look surgery gained its popularity, I believe, many years ago in Minnesota through Dr. Wangensteen's influence. In his hands, it was part of an elaborate protocol that was well thought-out in advance and, in many situations, the patient was either asymptomatic or in good general condition. Wangensteen and his co-workers merely wanted to go in and take a look. It is much easier to do an exploratory laparotomy when the patient is doing well than it is to do an elective craniotomy.

Walker: I agree. Second-look operations in the abdomen or staging laparotomies, as performed in Hodgkin's disease, are a totally different undertaking than a craniotomy. We would need an extremely good indication for that.

D'Angio: It is rare indeed for someone with medulloblastoma to succumb with either distant metastases or a spinal metastasis without a nidus of residual tumor at the primary site. I wonder if a second look might be built into some of the current studies as a planned procedure. In short, do these patients have residual disease, even with the best of radiation therapy to the primary site? Perhaps by monitoring with CAT and splitting the radiation therapy dose to avoid heavily irradiated areas of skin and brain, we might interpose a second look at a strategic point and determine whether the primary site could be brought under control by an additional debulking procedure.

Mahaley: Posterior fossa surgery is difficult enough the first time, without creating arachnoiditis and other problems that lead to shunts and general misery. A planned second look notwithstanding, the condition of the patient would be difficult for neurosurgeons to accept.

I would also like to add a word of caution concerning the medulloblastomas that are being entered into the chemotherapy protocols. Some neurosurgeons still believe that a significant enough number of these children respond well to operation plus radiotherapy. For this reason, they will hesitate before entering the children in a chemotherapy program within 3 weeks of operation rather than at the time evidence of recurrence is obtained.

Lane: I was responsible for design of the Southwest Group medulloblastoma study. Neurosurgeons may be satisfied with 5-year survivals of 50% in a tumor that may do a lot better, but that is not a satisfactory survival in terms of current pediatric oncology. I believe that these patients should be treated, particularly to prevent spinal metastases.

Mahaley: I also believe they should be treated, but I am not sure they should receive chemotherapy from the time of operation.

A. Raged: We are now in the same position with brain tumors that we were in with solid tumors in children 6 or 7 years ago. I also believe chemotherapy should be given to patients with medulloblastoma before they develop recurrent disease. That is the only way chemotherapy can be effective.

Evans: Someone has quoted a 5-year survival of 50% for childhood medulloblastoma; I know of no such figure. It must be a highly select group because it is extraordinarily good. The average medulloblastoma figures are not nearly that good. Once you start to treat recurrences, cure is uncommon. I believe that the principal role of chemotherapy is to prevent recurrence. We try a little bit of intratumor thio-TEPA rather than use chemotherapy intensively from the beginning. The neurologists have used dexamethasone for the last 15 or 20 years, but it is expensive and probablv is not any better than prednisone. The data Dr. Walker has shown suggest that we are 20 years behind the times. We should have started treating brain tumors with chemotherapy in 1956 when most of the drugs we now have, except the nitrosoureas, were available.

Kramer: You can get consistently a 5-year survival of 50% in patients with medulloblastomas treated adequately with radiation therapy, i.e., with a high dose to the whole cerebral spinal axis. This rate is not achieved in many places, and of course, the results are often worse. Clearly, a patient may die before he can be treated. We succeed in 50% of the cases, but we fail in 50%, and that is an unacceptably high rate. I agree that chemotherapy must be given a trial; however, it should be tried in a controlled fashion. If we are going to give everybody chemotherapy (at this point we do so without knowing the price), these children will pay. Therefore, and I say this with conviction, doing a randomized control trial is ethical, and it would be unethical to take any other course.

Walker: We are faced with such short survivals in many cases and such devastating recurrent disease that I must agree. The data from solid tumors derived by Skipper and Schabell and many other people indicate that the tumor is most sensitive and most responsive to radiotherapy and chemotherapy when the tumor burden is at its smallest. We need to approach these tumors early but in a controlled way, and the same thing applies to malignant gliomas. I am quite dissatisfied with the concept of waiting until the patient has a recurrence before starting to treat, because at that time you have to deal with a large tumor burden.

D'Angio: One of the major landmark series concerning survival after radiation is that from Manchester that has been quoted as a 5-year survival of 40%, but actually, that is a select group of patients. If you take the entire group who entered that system of hospitals, the 40% applies only to those who completed the course of radiation therapy. Hence there is a considerable decrease on the way; there are not immediate postoperative deaths, but simply patients who have not been able to get through the total course of radiation therapy. In any case, even 40% is not good enough, and we must also address ourselves to the *quality* of survival. We cannot ignore what happens to these children after treatment A versus B, even though the survival results may be precisely the same. There is no doubt that irradiating the entire CNS in children has profound long-term effects. Not only does it stunt growth, but, as the Manchester data show, it has severe effects on the brain. Many of these children are impaired, even though they survive into adulthood. Therefore, we must consider the quality of survival, even with the same 5-year survival rate. At the moment, we have not encountered any direct long-term effects on the CNS of the chemical agents now being employed. That is not to say that the chemical agents do not have these effects (indeed they may be worse) and should not be investigated, but these are basic considerations.

W. Cheek: I am a neurosurgeon who deals primarily with the pediatric age group, and I would like to congratulate Dr. Evans. I think neurosurgeons have never cured a medulloblastoma without using other forms of therapy. I believe the therapy record to date is not good, even with radiation, and agree that we should all look at medulloblastoma as a non-neurosurgical disease.

Hildebrand: I would like to be in the position in which those treating leukemia are, and hence I believe that chemotherapy should be given immediately after operation. However, in the instances when chemotherapy is effective in controlling residual disease (80% in breast cancer and probably more in leukemia), there are agents that induce a high percentage of objective remission. In the types of tumors we are treating, including medulloblastoma or glioma, we do not have an agent or agents that will induce a high rate of objective remission. Thus the chances to eradicate residual disease by chemotherapy are probably low. Would you not agree?

Raged: That may not be true. None of the drugs was effective in treating osteogenic sarcoma because we waited until metastasis developed before treating it. Each single agent gives a 10% response rate, but when these are combined, we get a 70% tumor-free survival with at least 1.5 or 2 years median survival. So if marginally effective drugs are given before there is metastatic disease, they may be effective.

Evans: At Children's Hospital in Philadelphia, we treated a small series of patients for recurrences of high-grade astrocytomas, glioblastomas, and medulloblastomas. We have a 50% rate of objective remissions with vincristine; 5 of the 20 children with recurrent tumors are long-term survivors after treatment with vincristine alone.

Lane: Dr. Hildebrand referred to the fact that we have other agents to induce remissions in leukemia. We now need phase II studies to evaluate possible agents for use in brain tumor therapy because an insufficient number of drugs are available for clinical use.

Walker: I have to agree with you. During the year spent in planning the protocol and in obtaining approval for a study of L-phenylalanine mustard, more than 10,000 women died from breast cancer. We could have learned a great deal from them, yet we wasted much time in discussion; many lives and a substantial amount of information could have been saved.

C. Chang: I would like to ask about the design for the study of medulloblastoma ependymoma. I would emphasize stratification. The protocol does not seem to have been stratified for stage of the disease; again, we are talking about the bulk of the tumor. Medulloblastoma T-1 survival rates are definitely superior to T-3 and T-4, and we are also talking about spinal metastases. We analyzed our data after 5 years, and our rate for T-1 medulloblastoma now is a 5-year survival of 70–75% versus zero for T-4. Does the protocol contain any stratification of cases?

Lane: The only stratifications in that protocol at the moment are for age and for placement of a shunt.

Chang: In the more recent studies, we did put that in a stratification. I think medulloblastoma is different from ependymoma.

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ara-C	Cytosine arabinoside
ASV	Avian sarcoma virus
atm	Atmospheres
AV	Arteriovenous
BCNU	1,3-bis(2-chloroethyl)-1-nitrosourea
BG	Basal ganglia
BK	Human papovavirus BK
BTSG	Brain Tumor Study Group
CAT	Computerized axial tomography
CCNU	1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea
CCT	Computerized cranial tomography
CNS	Central nervous system
CSF	Cerebrospinal fluid
CTT/CT	Computerized transaxial tomography/computerized tomography
DMBA	7,12-dimethylbenz[*a*]anthracene
ECF	Extracellular fluid
EEG	Electroencephalogram
EMI	Electronic Medical Instruments
ENU	Ethylnitrosourea
FMF	Flow microfluorometer(metry)
FSD	Focal spot to skin distance
GF	Growth factor
GFA	Glial fibrillary acidic
GI	Gastrointestinal
H&E	Hematoxylin and eosin
HPO	Hyperbaric oxygen
ILS	Increased life-span
keV	Kilo electron volt
kVp	Kilovolt peak
Ki-MuSV	Kirsten strain murine sarcoma virus
LiF	Lithium fluoride
Me-CCNU	1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
MMS	Methylmethanesulfonate
M-MuSV	Moloney murine sarcoma virus
MNU	Methylnitrosourea
MST	Median survival time

MTX	Methotrexate
MuSV	Murine sarcoma virus
OER	Oxygen enhancement ratio
PAS	Periodic acid–Schiff
PCH	Polycyclic hydrocarbons
PCNU	1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea
PCZ	Procarbazine
PDP	DNA polymerase activity
PETT-III	Positron emission transaxial tomography
PML	Progressive multifocal leukoencephalopathy
PNS	Peripheral nervous system
R	Roentgen
rCBF	Regional cerebral blood flow
ret	Rad equivalent therapy
RSV	Rous sarcoma virus
SA7	Simian adenovirus 7
SSV	Simian sarcoma virus
SV40	Simian virus 40
TAG	TARGET-attacking globulins
TARGET reagent	Topographic antigen-like reagent templates
thio-TEPA	Triethylenephosphoramide
TIW	Treatments per week
TLD	Thermoluminescent dosimeter
TMS	Trimethylsilyl ether
VER	Visual evoked response
XRT	X-ray therapy

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